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Identification of Larvae of Australian Baetidae

J.M. WEBB[1,2,3] AND P.J. SUTER[1,2,4]

[1] La Trobe University, Department of Environmental Management and Ecology, Wodonga, Victoria, Australia.
[2] Taxonomy Research Information Network (TRIN) http://www.taxonomy.org.au/
[3] JMW975@yahoo.com
[4] p.suter@latrobe.edu.au

Abstract

Identification keys are provided for larvae of 57 species of Baetidae from Australia for the genera *Bungona* (2 spp), *Centroptilum* (10 spp), *Cloeon* (7 spp), *Offadens* (32 spp), *Platybaetis* (1 sp), and *Pseudocloeon* (5 spp). Species concepts were formed by using a combination of morphological characters and nuclear and mitochondrial DNA sequences. The total number of Baetidae species in Australia isn't yet known as five species of *Centroptilum* and *Cloeon* known from adults have not yet been associated with larvae.

Introduction

The Baetidae is the largest family of mayflies, with over 900 described species and 100 genera globally (Gattolliat and Nieto, 2009). The first baetid described from Australia was *Baetis soror* Ulmer from Western Australia. Prior to the 1980s virtually all baetids were placed into the genera *Baetis* Leach, *Centroptilum* Eaton, *Cloeon* Leach, and *Pseudocloeon* Klapálek, and the same pattern was followed in Australia. In recent decades, however, all of these genera have been found to be artificial and polyphyletic and numerous new genera have been established.

The most significant contribution to the knowledge of Australian Baetidae is the identification keys produced by Suter (1997), where 9 genera and 23 morphospecies of running water taxa were treated. Prior to this, only 13 species were known from Australia: *Baetis baddamsae* Harker; *B. confluens* Harker; *B. frater* Tillyard; *B. soror* Ulmer; *Bungona narilla* Harker; *Centroptilum collendum* Harker; *C. elongatum* Suter, 1986; *Cloeon fluviatile* Ulmer,; *C. nandirum* Harker; *C. paradieniense* Suter; *C. tasmaniae* Tillyard; C. *virens* Klapálek; *Pseudocloeon kraepelini* Klapálek.

Since 1997, several species and genera were described by Lugo-Ortiz and McCafferty from Purdue University, USA. *Offadens* Lugo-Ortiz & McCafferty was established for *O. sobrinus* Lugo-Ortiz & McCafferty and *O. soror* (Ulmer), and *Edmundsiops* Lugo-Ortiz & McCafferty was established for *E. instigatus* Lugo-Ortiz & McCafferty. *Cloeodes fustipalpus* Lugo-Ortiz & McCafferty and *Cloeodes illiesi* Lugo-Ortiz & McCafferty were described from a small series of specimens, but were later synonymised with *Bungona narilla* (Suter and Pearson 2001).Three species of *Pseudocloeon* were also described by Lugo-Ortiz *et al.* (1999): *P. hypodelum* Lugo-Ortiz & McCafferty; *P. inconspicuum* Lugo-Ortiz & McCafferty; *P. plectile* Lugo-Ortiz & McCafferty. None of the species described by Lugo-Ortiz and McCafferty were compared to the morphospecies identified by Suter (1997).

Suter also described two species since 1997: *Platybaetis gagadjuensis* Suter from the Northern Territory (Suter 2000), and *Edmundsiops hickmani* Suter from eastern Australia. *Bungona narilla*, *Cloeon tasmaniae*, and *Offadens frater* were redescribed (Suter 2000, Suter and Pearson 2001).

The current study increases the total number of Baetidae larvae known from Australia to 57 species, although the number of species can vary depending on interpretation of the molecular data. Five species are known only from adults and are not included in the keys. It is likely these species are conspecific with undescribed species already included.

Some parts of this study are still preliminary, and the specific status of some groups remain unclear. Many taxa have been identified as being genetically distinct, but diagnostic morphological characters remain elusive. In some cases, the adult stages differ among cryptic larvae.

This study re-examines the status of Australian baetid species and genera and provides identification keys for species from all genera. Species limits and generic concepts were based on a combination of molecular, morphological, and biogeographical data. Whenever possible, voucher codes established by Suter (1997) have been retained or updated to reflect currently named species and genera.

Morphological Characters Used in Keys to Baetidae

Mouthparts

The various mouthparts are useful for generic differentiation and in some genera, species differentiation. An overview of the different mouthparts is provided in Figure 1. The labrum is the anterior most mouthpart, followed posteriorly by a pair of mandibles, a hypopharynx, a pair of maxillae, and a labium.

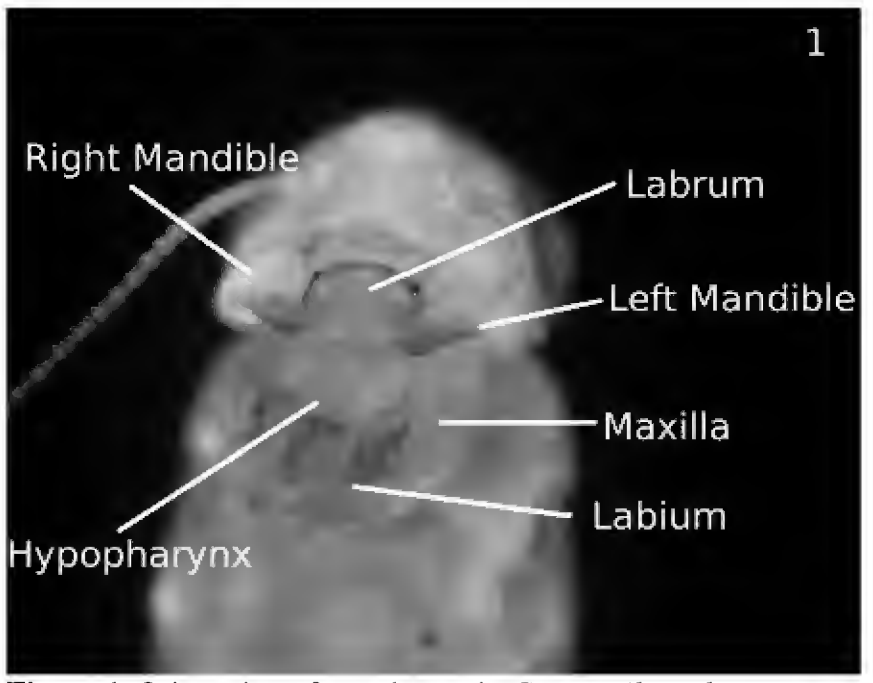
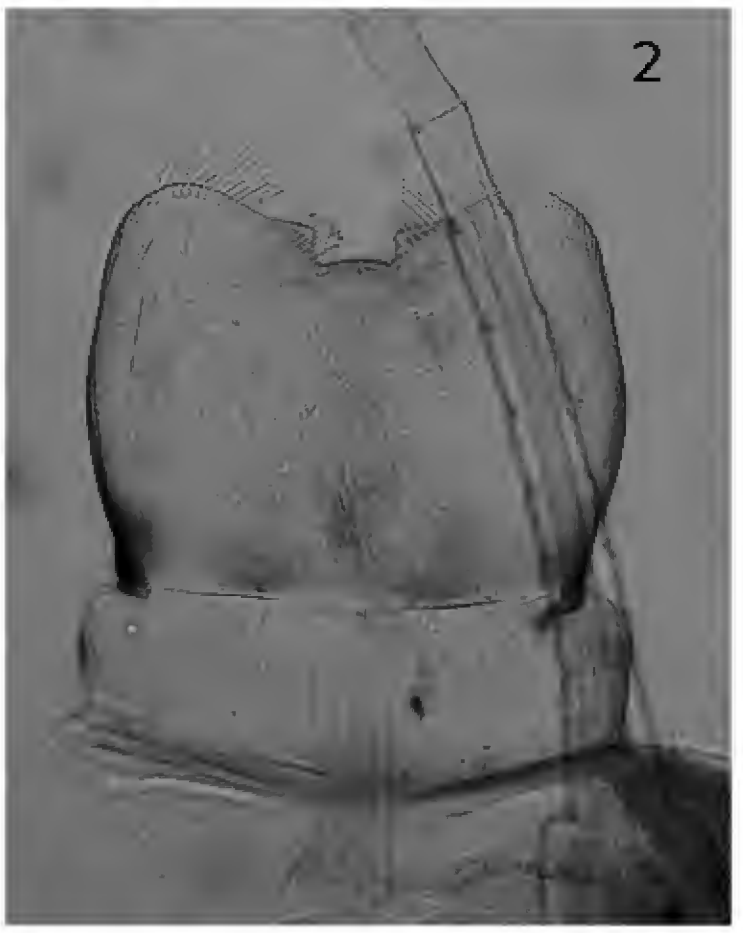
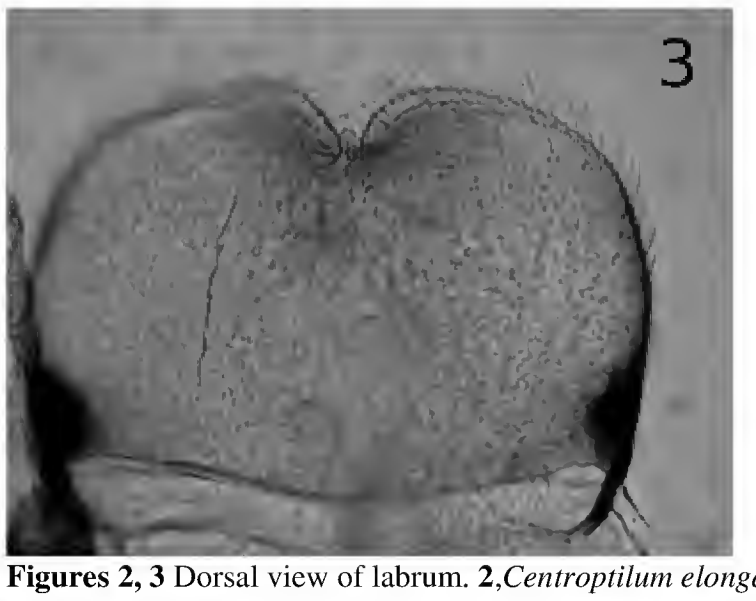


Figure 1. Orientation of mouthparts in *Centroptilum elongatum.*

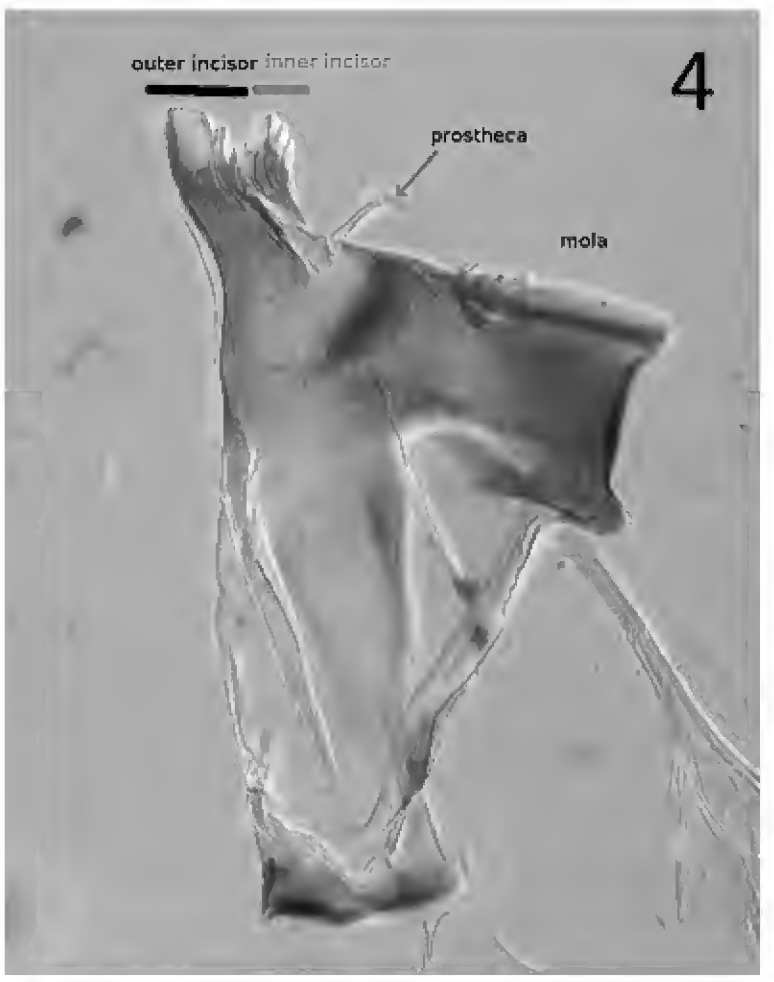
The anterior margin of the labrum generally has a small median notch (Fig 3), but in *Centroptilum* the whole of the anterior margin is excavated and the medial notch has a small pair of tooth like projections (Fig 2).

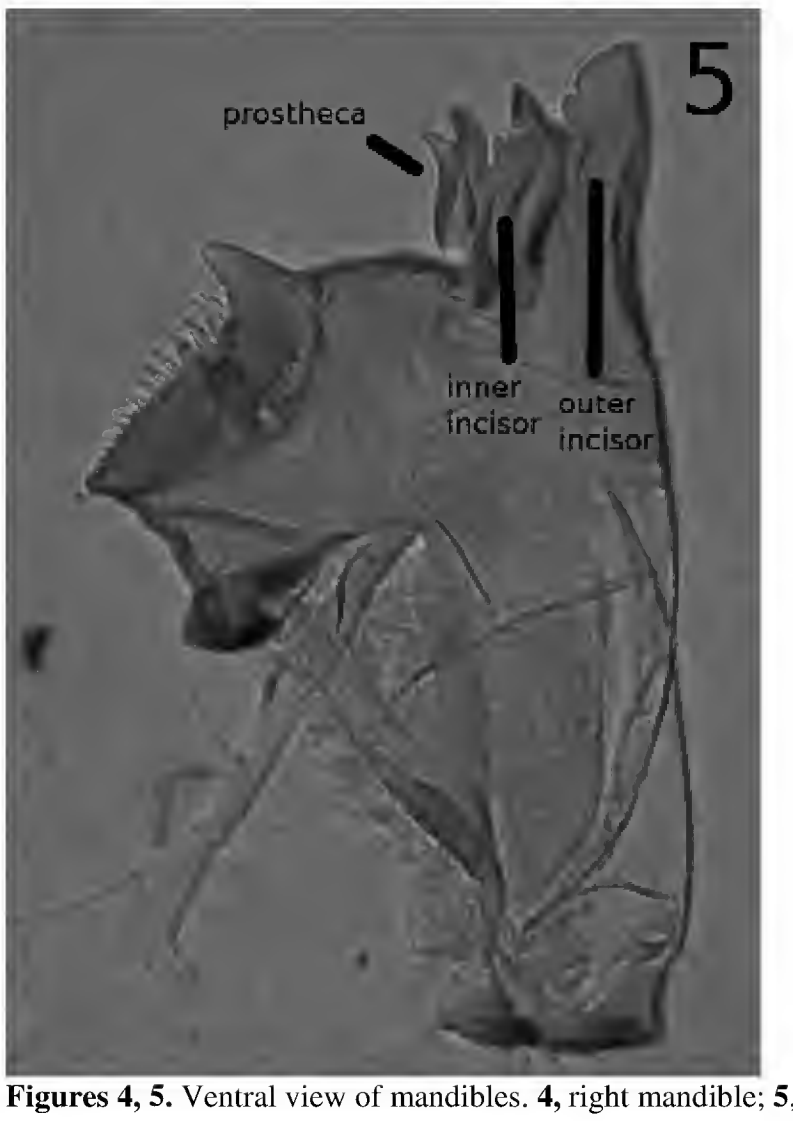




Figures 2, 3 Dorsal view of labrum. **2**,*Centroptilum elongatum* ; **3,** *Pseudocloeon inconspicuum.*

The mandibles consist of an outer and inner pair of incisors, a small prostheca attached near the base of the inner incisor, and a mola. The right (Fig 4) and left (Fig 5) mandibles are similar in structure, but differ in the orientation of the molars, with the right mandible have a flat or planate molar, and the left mandible having an angulate molar. Examining the area between the molar and prostheca and determining whether the prostheca is forked (Fig 6) or simple on the right mandible is essential for differentiating species in *Offadens*. The incisors can be variously fused, and the number of apical teeth and the presence (Fig 6) or absence of a small lateral tooth are useful for differentiating some species.





Figures 4, 5. Ventral view of mandibles. **4,** right mandible; **5**, left mandible.

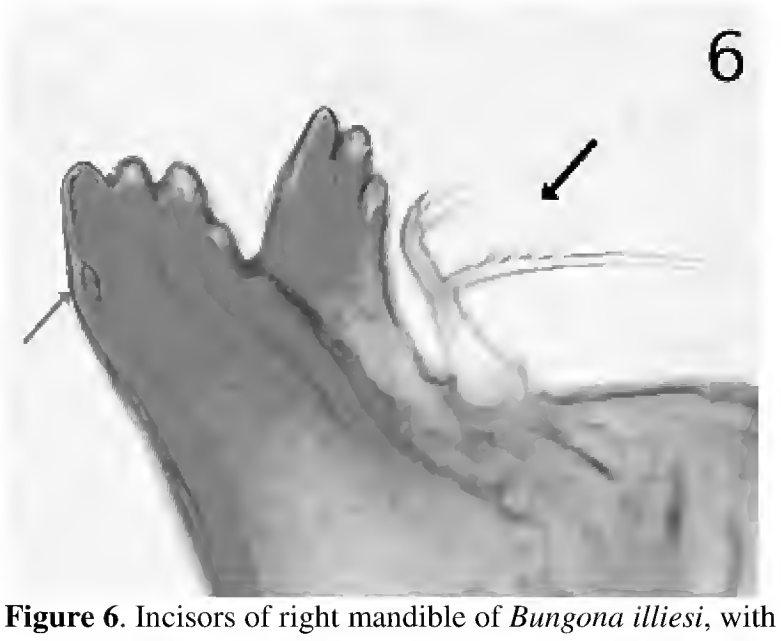


Figure 6. Incisors of right mandible of *Bungona illiesi*, with a lateral tooth on the outer incisor (left arrow), and a forked prostheca (right arrow).

The maxillae (Fig 7) are composed of a two or three segmented palp, a fused galealacinia, and a crown of several rows of setae and and four apical denticles/spines. At the base of the galealacinia is generally a small row of basal setae and a single median hump seta. The right and left maxillae are usually identical.

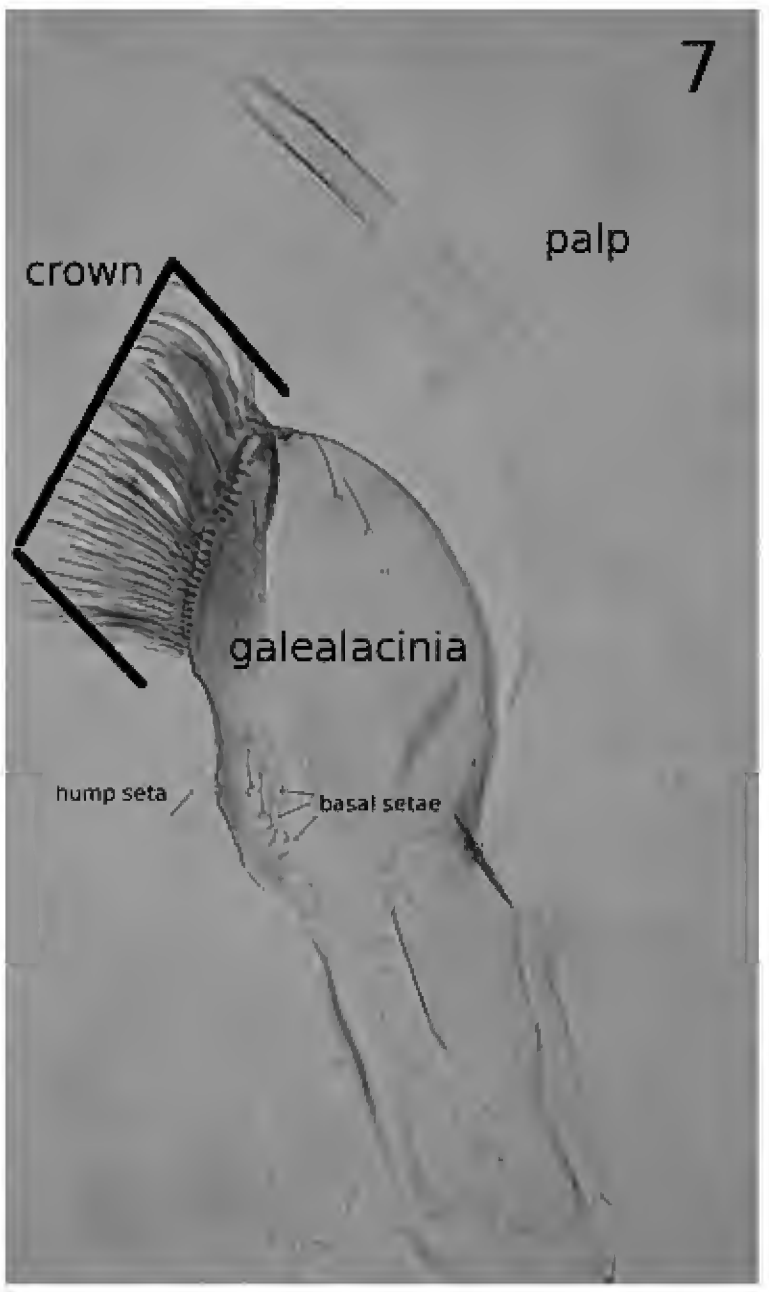


Figure 7. Maxilla of *Centroptilum* sp.

The hypopharynx (Fig 8) is located in the middle of the other mouthparts. Its shape can be of phylogenetic significance, but it generally isn't used diagnostically.

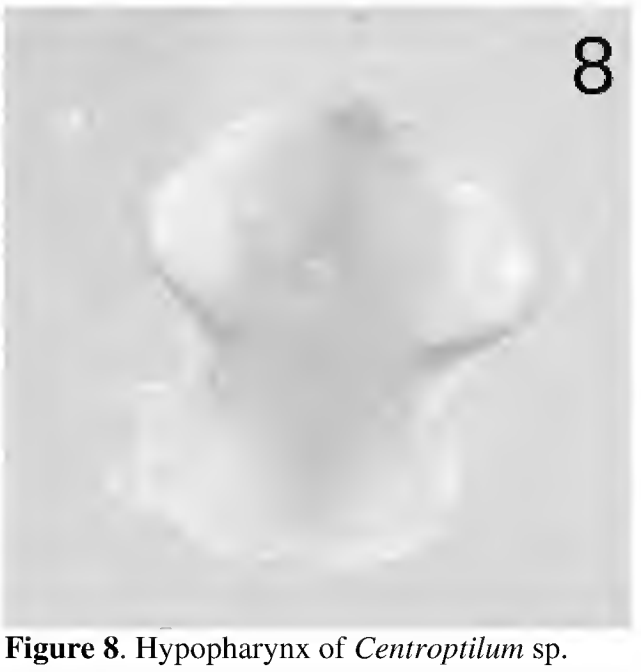


Figure 8. Hypopharynx of *Centroptilum* sp.

The labium (Fig 9) consists of a pair of three segmented palps, an outer pair of paraglossae, and an inner pair of glossae. The shape of the palps, particularly the medial development of segment two, can be a useful character for diagnosing some species. The relative width of the glossae and paraglossae are useful for identifying genera such as *Pseudocloeon* and *Platybaetis*.

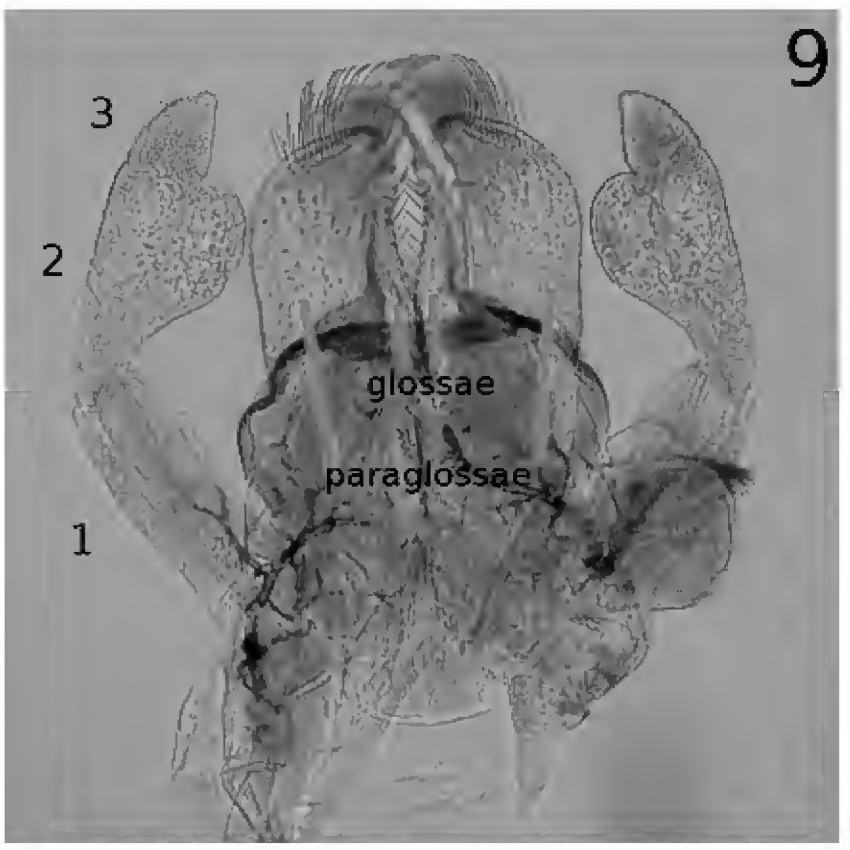


Figure 9. Labium of *Pseudocloeon inconspicuum* with palp segments numbered.

Legs

The legs consist of a basal coxa, trochanter, femur, tibia, tarsus, and a tarsal claw (Fig 10). The coxa and trochanter are generally not useful for diagnosis, but the other segments of the legs are relied upon heavily in these keys. The length of the claws relative to the tarsi can be diagnostic for some genera, as can the shape of the claw. The femur (Fig 11) has a dorsal row of setae which can vary in size, shape and number. Near the apex of the femur is usually a pair of medium to large subapical robust setae (Figs 11 & 12), and the rounded apex of the femora has several robust setae of varying size and usually some scattered fine setae (Fig 12). The anterior surface (Fig 11) of the femur has several small to medium robust setae of varying shape and frequently has fine setae as well. The ventral margin of the femur has few to many short robust setae of varying shape and sometimes has

numerous fine setae as well. Setae are generally absent on the posterior surface of the femur, except in *Cloeon*.

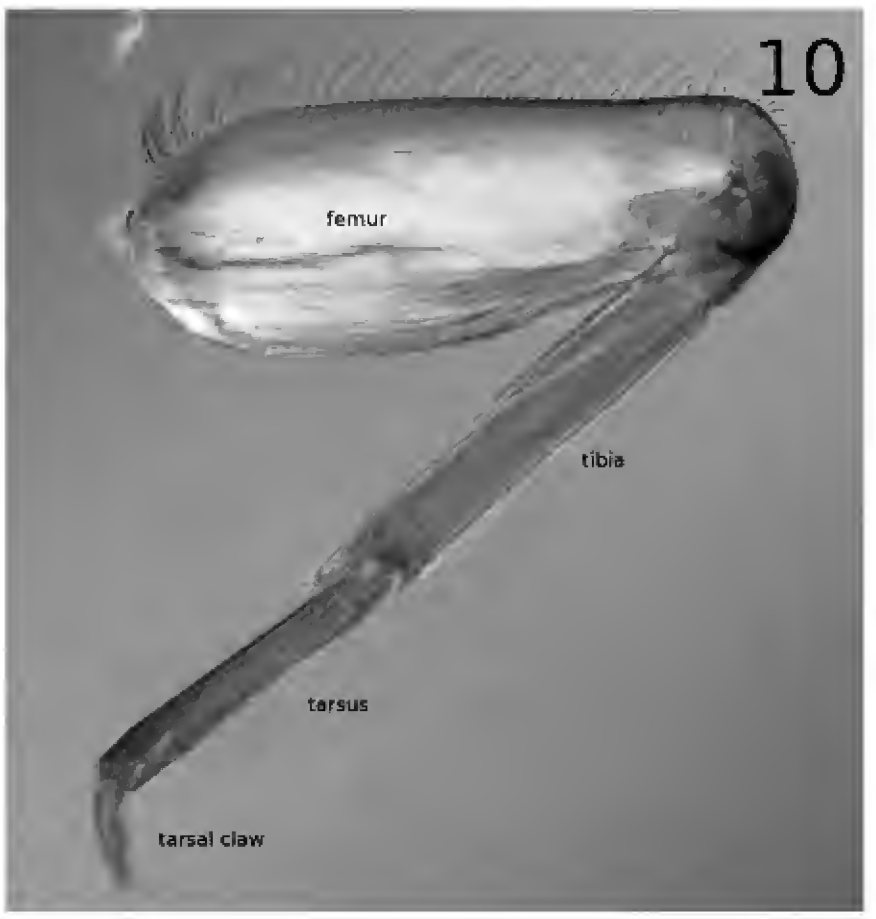


Figure 10. Leg segments.

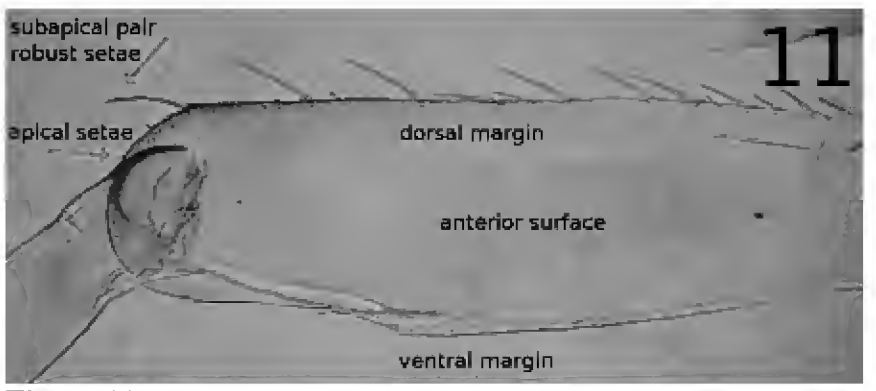


Figure 11. Femur.

Figure 12. Apex of femur.

The tibia has several characters of taxonomic importance. The outer margin usually has a row of robust setae of varying size and shape, and near the apex of the outer margin is usually a distinct robust seta. In some species the subapical seta is extremely long (as in Fig 13), but in most species it is a moderate length. A few species lack the subapical seta completely or its presence is unclear due to the presence of a very long row of robust setae on the outer margin. The inner margin generally has numerous robust setae in scattered rows.

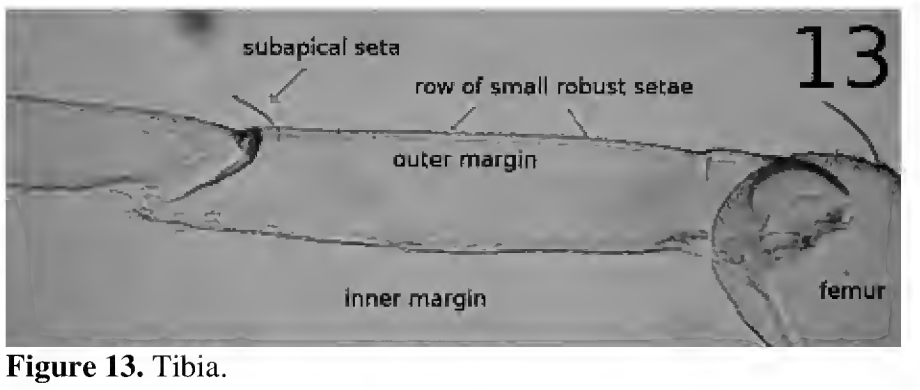


Figure 13. Tibia.

The tarsus (Fig 14) is of less use than the other leg segments for diagnosis. The tarsus generally has at least one inner row of robust setae, and the outer margin may have robust setae as well

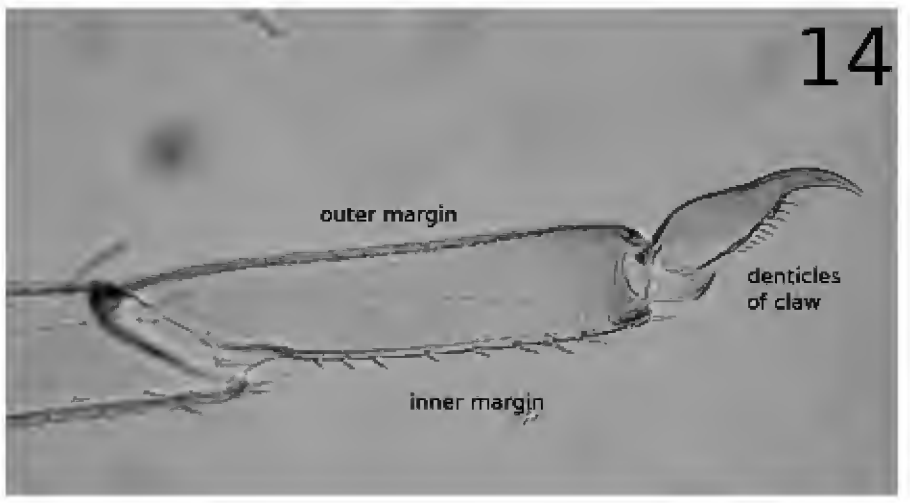


Figure 14. Tarsus and tarsal claw.

The tarsal claw bears either one or two rows of denticles. In some species there is a small subapical seta that is of taxonomic use. The seta, however, is often difficult to see and is prone to breakage. If it cannot be determined whether a seta is present, the leg may be placed on a microscope slide with a small amount of water or alcohol and a coverslip and examined with a compound microscope.

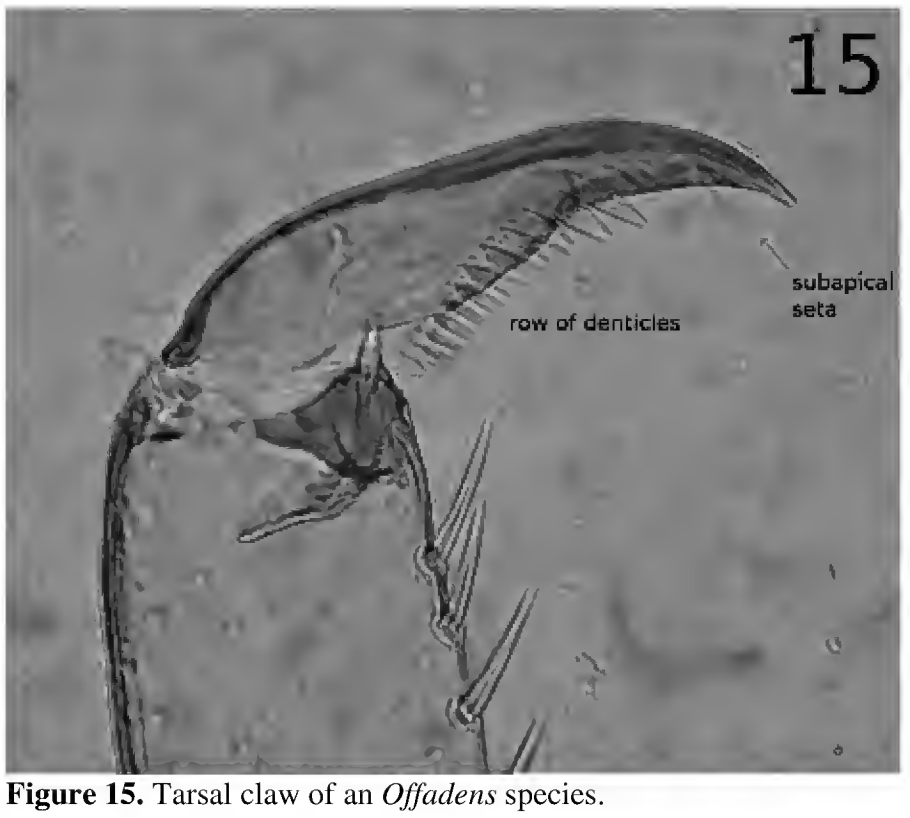


Figure 15. Tarsal claw of an *Offadens* species.

The thorax consists of three segments, the prothorax, mesothorax, and metathorax, the dorsal portions of which are the pro-, meso-, and metanota. The ventral portions of the thorax are the sterna. All species have a conspicuous pair of wingpads on the mesonotum (Fig 16). The size of the wingpads increases with every moult and in the last instar

they will be black and the actual wing can often be observed developing inside. Occasionally the wing can be teased out of the wingpad and very gently spread apart to observe the developing venation. The hind wingpads, if present, are much smaller than, and are hidden underneath, the fore wingpads, but can usually be easily observed in mid- to late instar larvae. It may be necessary to lift the fore wingpads to see the hind wingpads in some specimens.

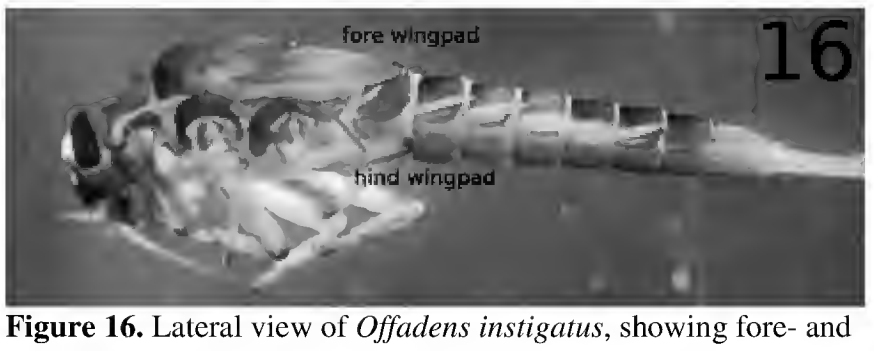


Figure 16. Lateral view of *Offadens instigatus*, showing fore- and hind wingpads.

The abdomen is composed of 10 segments, with the first segment adjacent to the thorax. Abdominal segments 1 or 2 – 7 have a lamella-like gill in the posterolateral corner. In all genera but *Cloeon*, the gill has a single plate-like lamella; in *Cloeon* segments 1 – 6 have two lamellae per gill and a single lamella on segment 7. Two genera, *Pseudocloeon* and *Platybaetis*, lack gills on segment 1. Gills are very prone to falling off specimens upon preservation, especially in higher concentrations of ethanol. An apparent absence of gill 1 should be checked by looking for a small sclerotised gill socket on the posterolateral corner.

The terga are the dorsal surfaces of the abdominal segments. The posterior margin of each tergite has a series of spines that can be of diagnostic use. The colour pattern on the terga of freshly collected specimens can be extremely useful for identifying species. In alcohol, however, the colour pattern tends to fade over time (the colour patterns will remain longer if the specimens are stored in the dark and in cool temperatures). Only nine sterna are apparent and the sternal surfaces are generally less useful taxonomically. At the apex of the abdomen is a pair of multisegmented cerci and a median caudal filament (vestigial in one Australian species). Collectively, the cerci and terminal filament are called the caudal filaments. At the base of the caudal filaments is a pair of paraprocts that have spines along their margins. Although the paraprocts can be of use for identifying species, they are not used in this key.

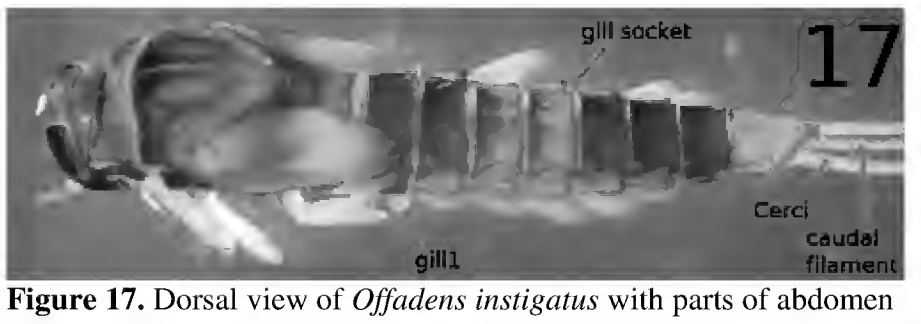


Figure 17. Dorsal view of *Offadens instigatus* with parts of abdomen labelled.

Setae and Spines

There is much variation in the terminology used for describing the various setae and spines on insects. We take the view that a spine is an immovable outgrowth of the cuticle. Spines that are useful for diagnosing species include those on the posterior margins of the terga and the row of spines (denticles) on the tarsal claws.

Setae are multicellular, hairlike sensory organs that have a basal socket and are extremely variable in size and shape. Fine setae are small, fine hairlike setae. Most setae are somewhat hairlike and are referred to as just setae. Robust setae are generally stout setae that tend to be slightly sclerotised, often have a median longitudinal rib, and vary greatly in shape.

Sexual Dimorphism in Larvae

Adult male baetids are unusual in having the compound eyes divided into two parts – a small 'normal' compound eye and a greatly enlarged 'turbinate' dorsal portion that is usually red or orange in colour. The turbinate eyes can be seen developing even in mid instar male larvae (Fig 18). Female larvae have only a 'normal' compound eye (Figs 16 & 18).

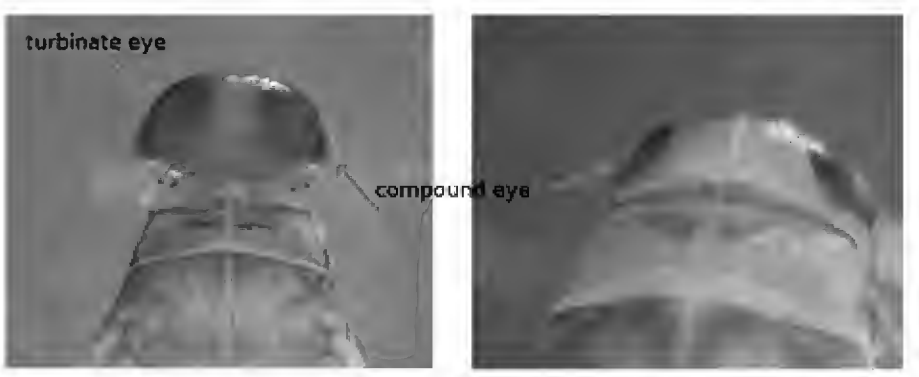
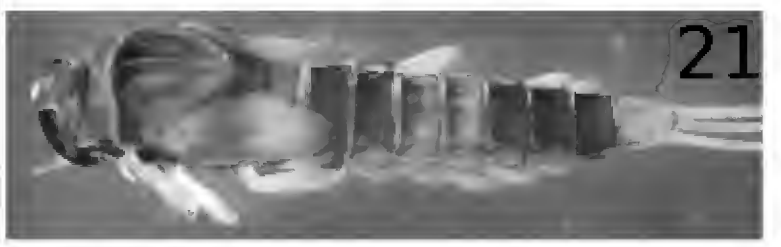


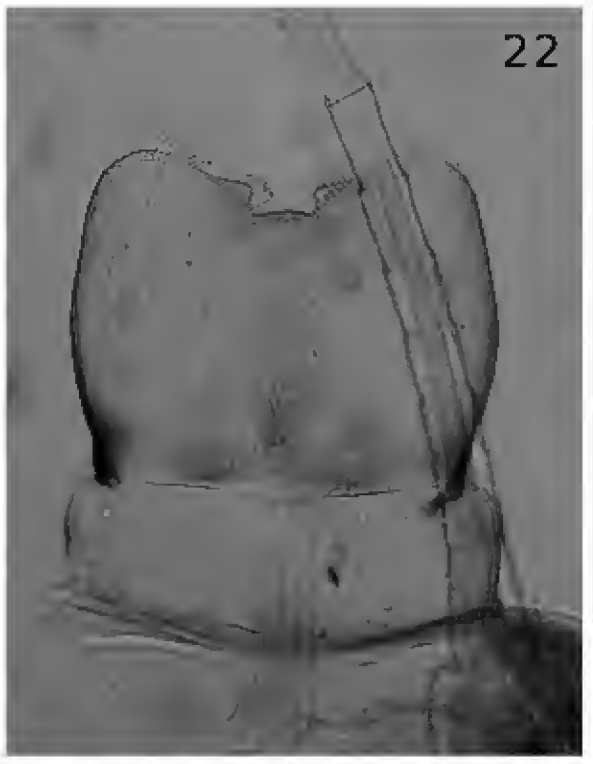
Figure 18. Eyes of male (left) and female (right) larvae of *Offadens* spARR1.

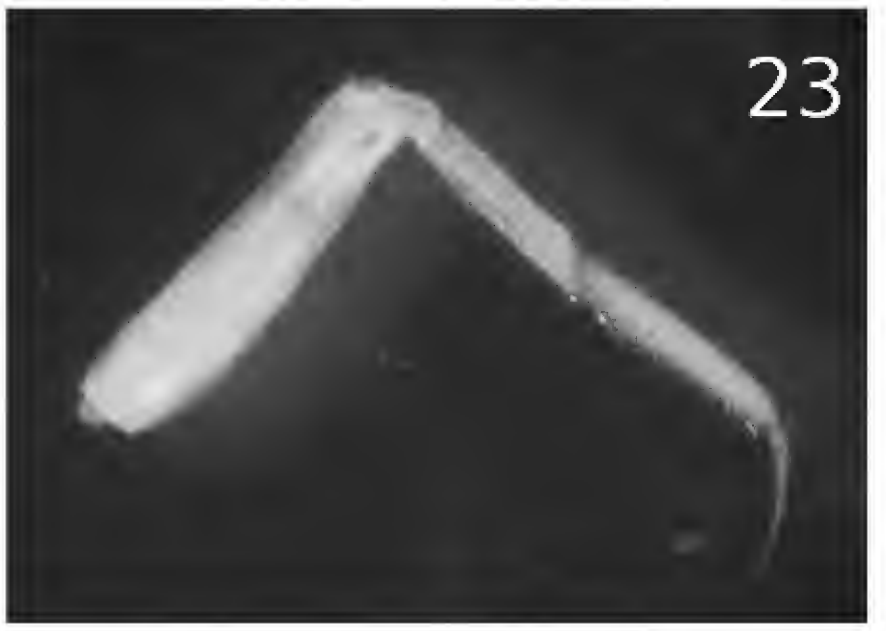
Generic Key for Larvae of Australian Baetidae

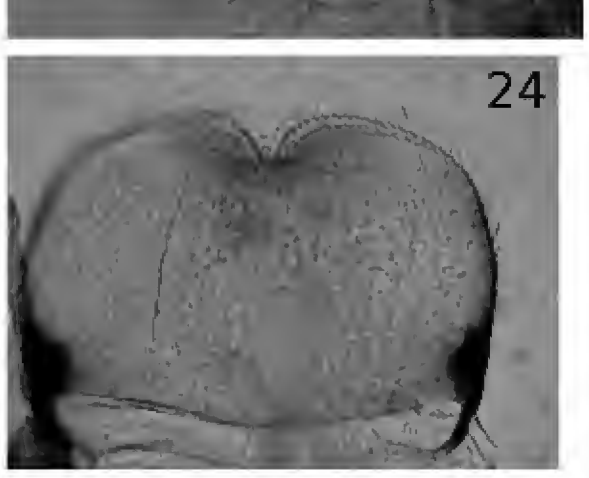
1a. Terminal filament vestigial (Fig 19); head distinctly pug-nosed (Fig 20); NT, N QLD .....***Platybaetis gagadjuensis*** **pg 21**
1b. Terminal filament well developed (Fig 21); head usually rounded in lateral perspective; widespread ….....**2**



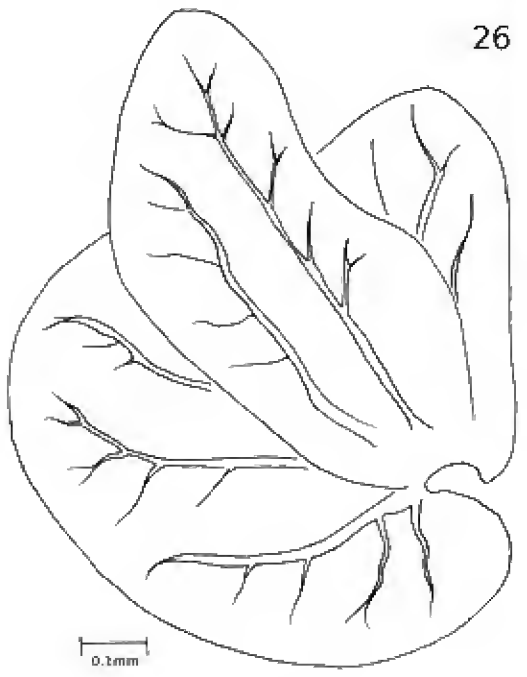
2a. Labrum with deep, wide notch (Fig 22); tarsal claws long and narrow with two rows of denticles, more than 0.6X length of tarsi (Fig 23) ….....***Centroptilum*** **pg 8**
2b. Labrum with small median U shaped notch (Fig 24); tarsal claws < 0.5X length of tarsi (Fig 25) ….....**3**

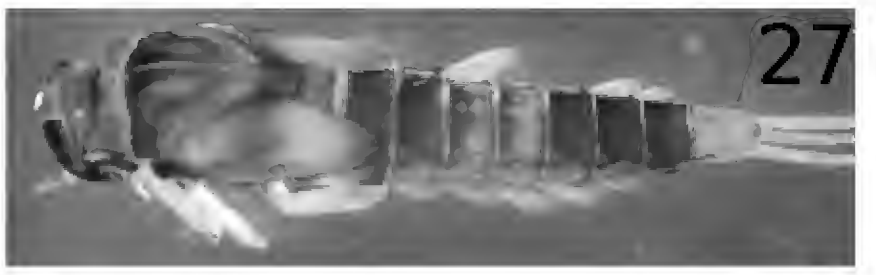




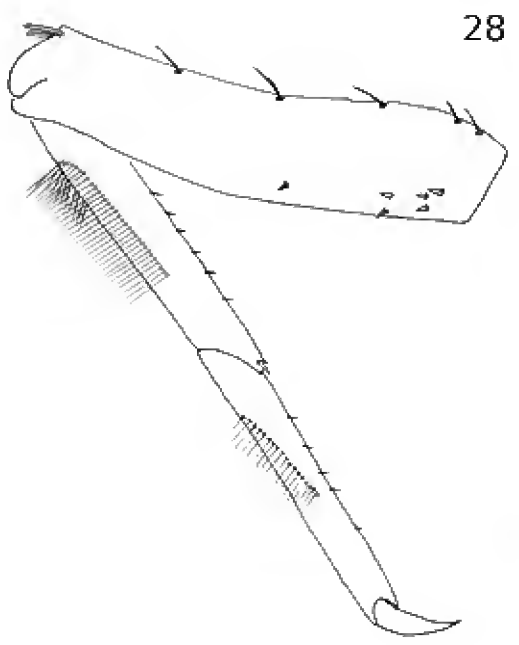


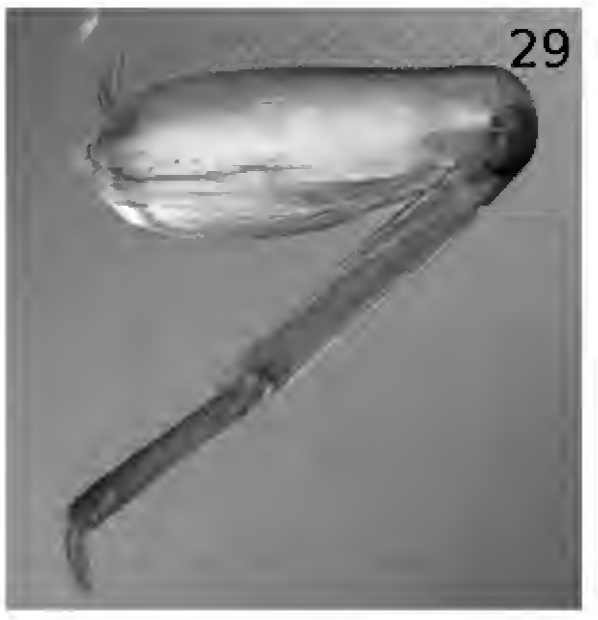
3a. Gills on abdominal segments 1 – 6 with double lamellae (Fig 26); hind wingpads absent; tarsal claws with two rows of denticles; abdomen with lateral spines on at least segmens 8-9 (see Figs 55, 56) .....***Cloeon*** **pg 10**
3b. All gills with single lamellae (Fig 27); hind wingpads present or absent; tarsal claws with one row of denticles; abdomen never with lateral spines ….....**4**



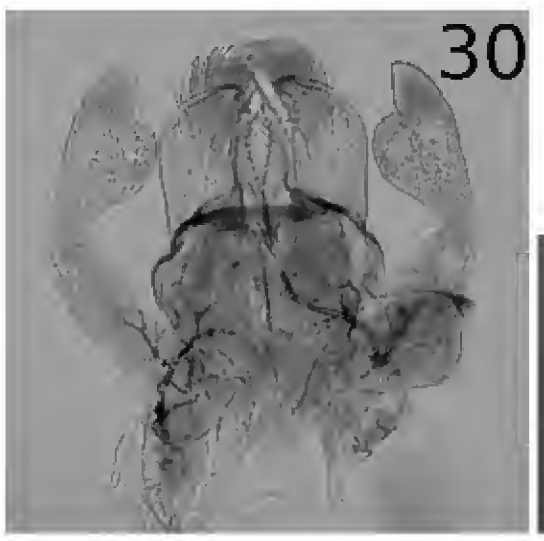


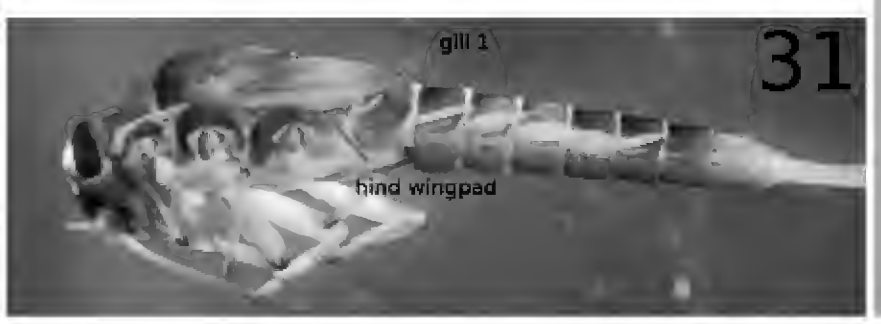
4a. Tibiae with extremely long row of fine setae that arcs over the outer margin onto posterior surface (Fig 28); tarsal claws without denticles; hind wingpads absent ….....***Bungona*** **pg 7**
4b. Tibia without an arcing row of fine setae, but outer margin usually with row of short robust setae (Figs 13, 25, 29); tarsal claws with denticles; hind wingpads present or absent ….....**5**





5a. Gills absent on segment 1; hind wingpads absent; paraglossae much wider than glossae (Fig 30) ……..***Pseudocloeon*** **pg 21**
5b. Gills present on segment 1 (Fig 31); hindwing pads present (Fig 31); glossae and paraglossae subequal in width (Fig 32) ……. ***Offadens*** **pg 13**





Bungona Harker

Diagnosis: Labrum with small U shaped notch; maxillae with two segments, sometimes appearing three segmented; glossae and paraglossae subequal in width; labial palps without median extension on segment 2; hind wingpads absent; tibiae with arc of very long fine setae; tarsi with arc of very long fine setae; tarsal claws with out denticles; abdominal sterna 4-6 with small tuft of sublateral fine setae; gills with single lamellae and present on segments 1-7; terminal filament subequal in length to cerci.

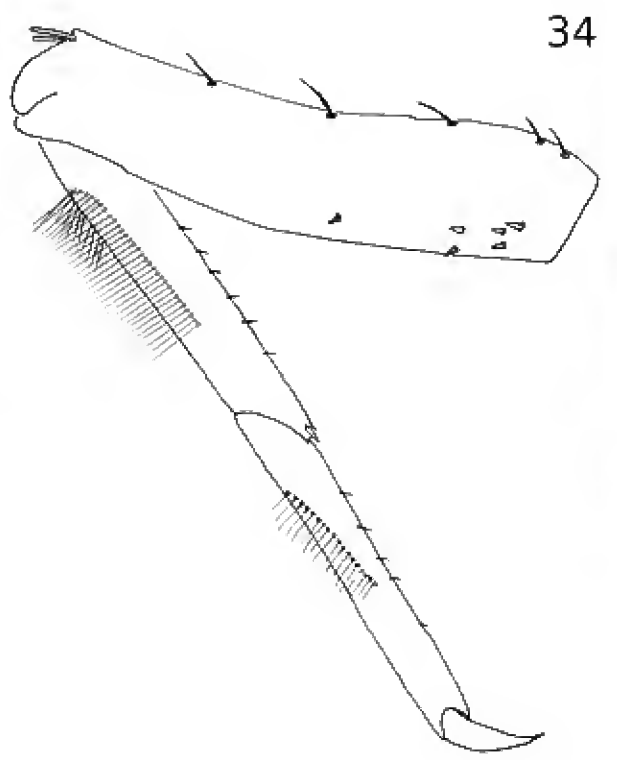
Distribution: North Queensland to Tasmania in running water.

Species Composition: *Bungona illiesi* (Lugo-Ortiz & McCafferty, 1998); *B. narilla* Harker, 1957.

Discussion: Lugo-Ortiz and McCafferty (1998) described *Cloeodes fustipalpus* Lugo-Ortiz & McCafferty and *C. illiesi* Lugo-Ortiz & McCafferty from a small series of larvae from northern NSW and northern QLD, respectively. Both were synonymised with *B. narilla* by Suter and Pearson (2001), but genetic evidence showed the northern QLD specimens to be genetically distinct from those from other parts of eastern Australia. We recently collected adults from northern QLD that were different from those of *B. narilla*, and there are small but consistent differences in the larvae. As such, Webb and Suter (2010b) removed *C. illiesi* from synonymy with *B. narilla* and treated it as a distinct species in the genus *Bungona*. A global revision is needed to clarify the relationship among *Cloeodes*, *Bungona*, and related genera.

Key to Species of *Bungona*

1a. North Queensland; mandibles with small lateral tooth on outer incisor (Fig 33); femora with relatively sparse and short robust setae near ventral margin (Fig 34); colour pattern variable, but often with most segments dark brown (Fig 35) ……..***B. illiesi***
1b. SE Queensland to Tasmania; outer incisors of mandibles without small lateral tooth; femora usually with longer and more dense robust setae near ventral margin (Fig 36); colour pattern usually more contrasting (as in top right specimen of Fig 35) ……..***B. narilla***



Centroptilum Eaton

Diagnosis: Labrum with deep V shaped notch with basal pair of small denticles; maxillae three segmented; glossae and paraglossae subequal in width; hind wingpads present; tibiae and tarsi without arc of long fine setae; tarsal claws 0.6 – 0.9 X length of tarsi and with two rows of denticles; gills present on segments 1 – 7, gill 1 curved outwardly and apically pointed; terminal filament subequal in length to cerci.

Distribution: ACT, NSW, NT, SA, TAS, VIC. Occurs in sandy habitats in depositional areas of streams. *Centroptilum elongatum* tends to occur in lower elevation streams and rivers, whereas others such as the *C.* sp1 complex are often found in cooler, higher streams.

Species Composition: *C. elongatum* Suter, *C. collendum* Harker, *C.* sp6, *C.* spARR, *C.* sp1, *C.* spLogan, *C.* spBlackstripe, *C.* spNQLD, *C.* spTAS, *C.* spSnowy, *C.* spWonnangatta, C. spANIC1.

Discussion: Both morphological and molecular analyses show that the Australian species are not congeneric with *Centroptilum*, but are rather more closely related to the new world genus *Callibaetis* Eaton. A new genus is in the process of being described (Webb, Gattolliat, and Suter, in prep.).

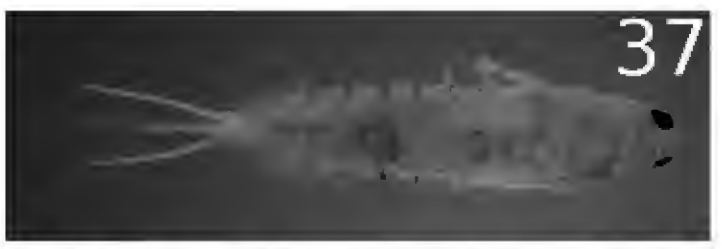
Centroptilum spBlackstripe is only known from a single population in the Wimmera R. in SW Victoria and is sympatric with *C. elongatum* and highly similar morphologically. It is possible *C.* Blackstripe is a colour morph of *C. elongatum*, but we cannot test this until DNA becomes available.

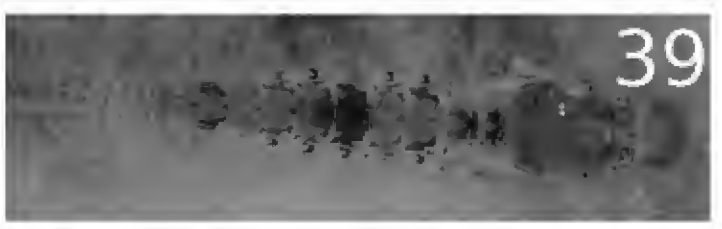
Centroptilum sp1, *C.* spTAS, and *C.* spSnowy are all morphologically cryptic but were identified as being moderately distinct with mitochondrial DNA. There are slight differences in colour pattern of the adults of *C.* sp1 and *C.* spTAS, but the adult of *C. s*pSnowy is unknown. It is possible this complex represents a single species, but until further information becomes available, we are treating them as distinct. *Centroptilum* Wonnangatta and *C.* sp6 are also morphologically similar to the *C.* sp1 complex, but genetically are highly distinct and belong to a different clade. The adults of both species are unknown, but we believe *C.* sp6 is equivalent to *C. collendum* based on distribution, and the adult of *C.* spWonnangatta may be the distinctive *C.* spANIC1 which has numerous red punctations on the abdomen.

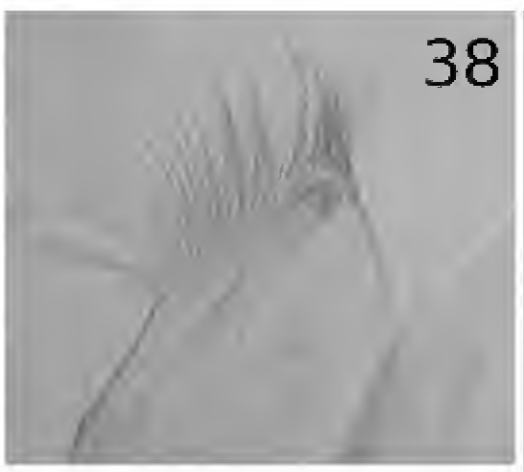
Because the larvae of *C.* spANIC1 and *C. collendum* are unknown or insufficiently described, they are not included in the key.

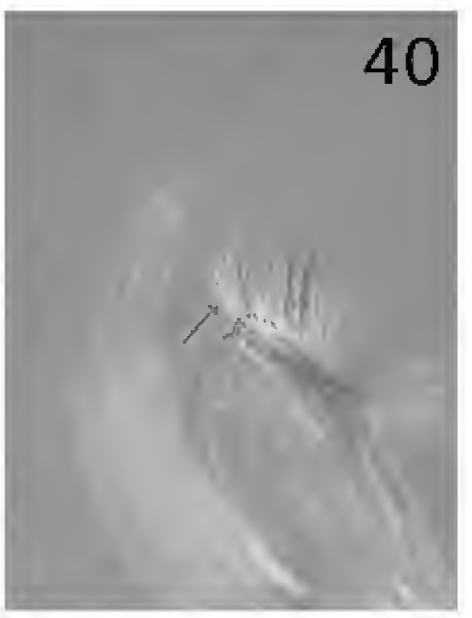
Key to Species of *Centroptilum*

1a. Northern Territory or northern Queensland; colour pattern with numerous minute black spots on terga (Fig 37); apical denticles on maxillae more robust than crown setae (Fig 38) **8**
1b. Elsewhere in Australia; colour pattern as in Fig 39; apical denticles on maxillae similar in size to crown setae (Fig 40) **2**



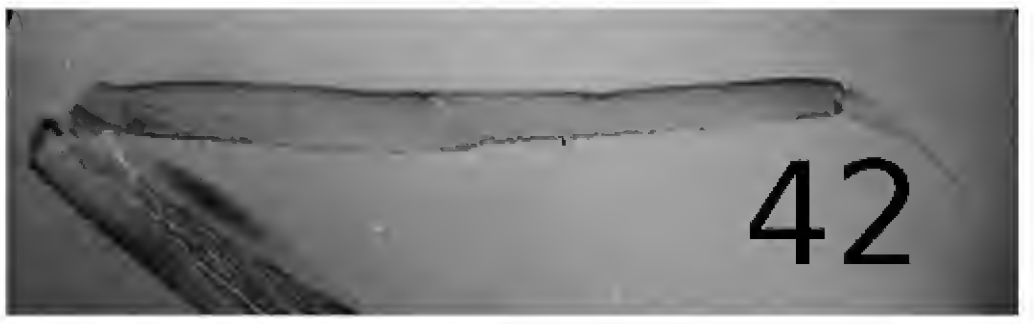




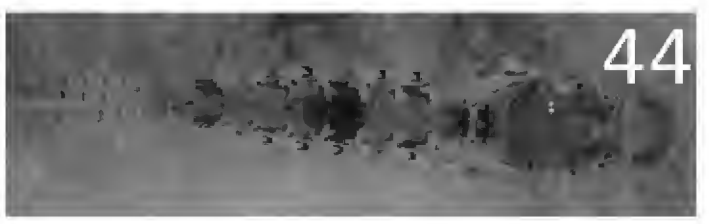


2a. Tasmania ….....*C.* **spTAS**
2b. South Australia ….....***C. elongatum*** **(in part)**
2c. Victoria, New South Wales, Australian Capital Territory, SE Queensland ….....**3**

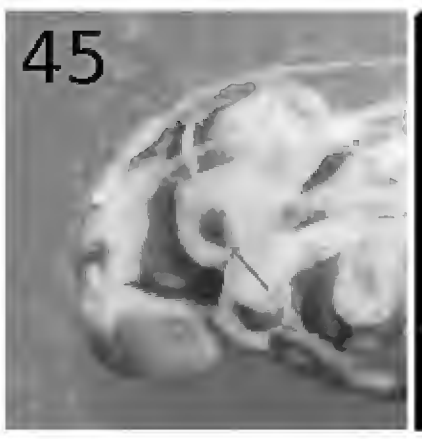
3a. Outer margin of tibiae with robust setae (Fig 41); subapical pair of setae on femur usually sharply pointed ….....**4**
3b. Outer margin of tibiae without robust setae (Fig 42); subapical pair of setae on femur usually short and blunt ….....**6**

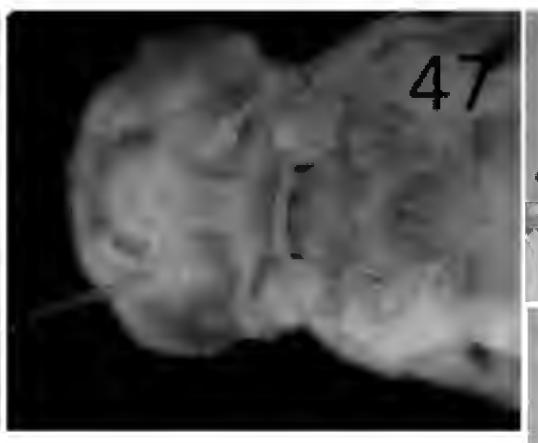


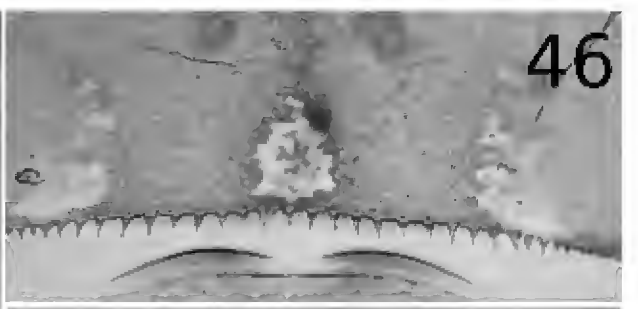
4a. Abdominal terga with distinct longitudinal black stripe (Fig 43); mesonotum with pair of submedian dark stripes (Fig 43); only known from the Wimmera River in SW Victoria .....*C.* **spBlackstripe**
4b. Abdominal terga without longitudinal black stripe (Fig 44); mesonotum without pair of sublateral dark stripes (Fig 44); SE Queensland to South Australia ….....**5**

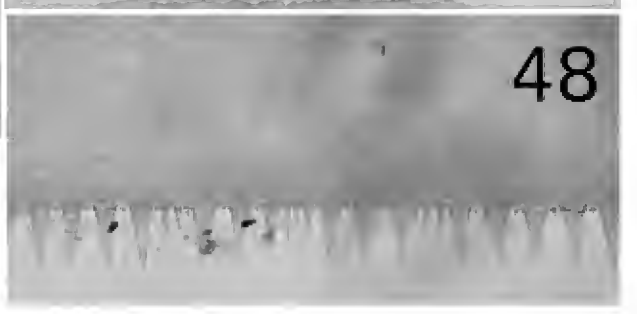


5a. Mandible with distinct black spot (Fig 45); maximum size 6.5 mm; posterior margins of terga with long widely spaced spines of equal size (Fig 46); SE Queensland, northern half of New South Wales ….....*C.* **SpLogan**
5b. Mandible without distinct black spot (Fig 47); mature larvae 8.0 – 10.0 mm; posterior margins of terga with spines closely together and of various sizes, usually alternating between long and short spines (Fig 48); South Australia to SE Queensland ….....***C. elongatum*** **(in part)**

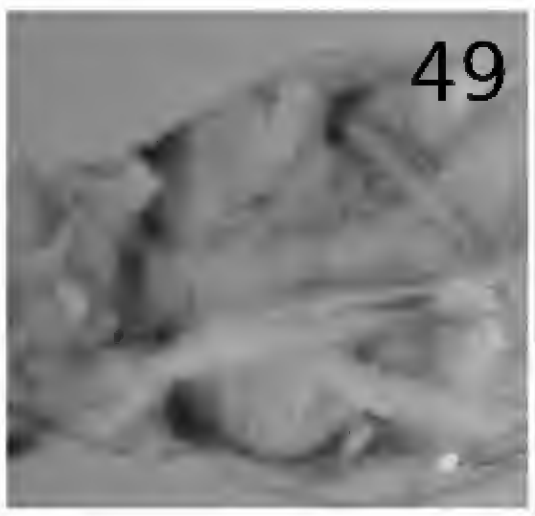


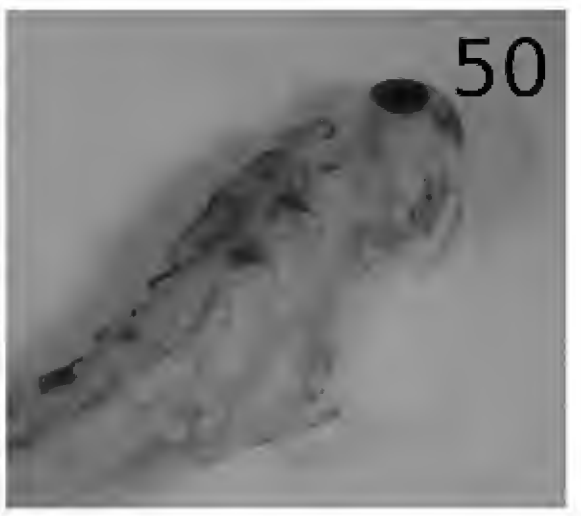






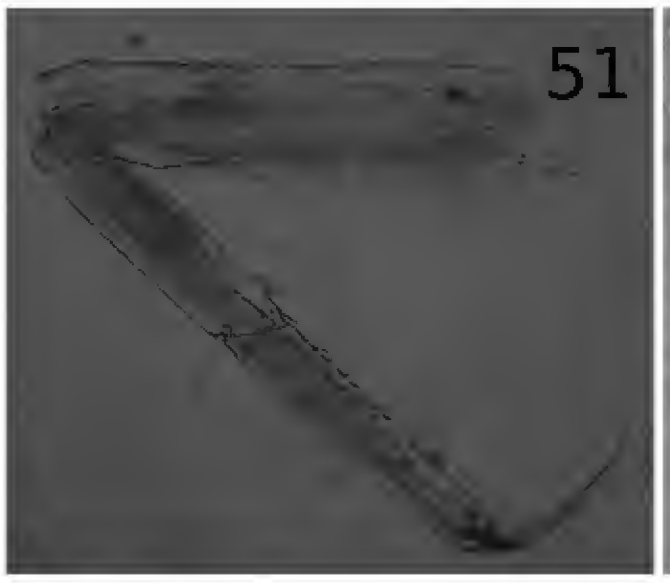
6a. Femora with distinct elongate spot subapically (Fig 49) known only definitively from the Wonnangatta River in Victoria, but based on presumed adult is present into northern New South Wales and probably SE Queensland ….....*C.* **spWonnangatta**
6b. Femora with indistinct subapical band (Fig 50); widespread in streams from Grampians and Otways to SE Queensland ….....**7**





7a. Spines present on posterior margins of sterna 6 or 7–9; sterna 2–8 with small sublateral black spot; mature size <7.2mm; eastern New South Wales .....***C.* Sp6**
7b. Spines present on posterior margins of sterna 4 or 5–9; sterna without sublateral black spots; mature size 8.0-10.0mm; widespread in streams from Grampians and Otways to SE Queensland .....***C.* sp1 and *C.* spSnowy**

8a. Northern Territory; robust setae on femora relatively long and numerous (Fig 51) .....***C.* spARR**
8b. Northern Queensland; robust setae near ventral margin of femora relatively short (Fig 52) .....***C.* spNQLD**



Cloeon Leach

Diagnosis: Labrum with small U shaped notch; maxillary palps two or three segmented; paraglossae and glossae subequal in width; hind wingpads absent; tibiae and tarsi with out long arc of setae; tarsal claws with two rows of denticles; gills present on segments 1 – 7, those on 1 – 6 with double lamallae; terminal filament subequal in length to cerci.

Distribution: Australia wide, primarily in still waters or depositional areas of streams. Larvae frequently occur in dense submerged vegetation. In the Northern Territory, some larvae were found to occur in rapidly flowing portions of streams over bedrock.

Species Composition: *C. fluviatile* Ulmer, *C. nandirum* Harker, *C. paradieniense* Suter, *C. tasmaniae* Tillyard, *C. virens* Klapálek, *C.* spRedStripe, *C.* spBlackSpot, *C.* spYellow, *C.* spNT2, *C.* spBigGill, *C.* spQ3.

Discussion: This treatment of *Cloeon* should be considered preliminary as some species may have wider distributions than indicated here, additional species may yet remain undiscovered, and some species concepts may require alteration as more DNA evidence becomes available.

In the Northern Territory and Queensland, adults of *C. fluviatile* are highly variable and may represent a complex of species; in other parts of Australia the adults are relatively uniform in appearance. Additional sequencing of these specimens should assist in clarifying the status of the variants of *C. fluviatile*.

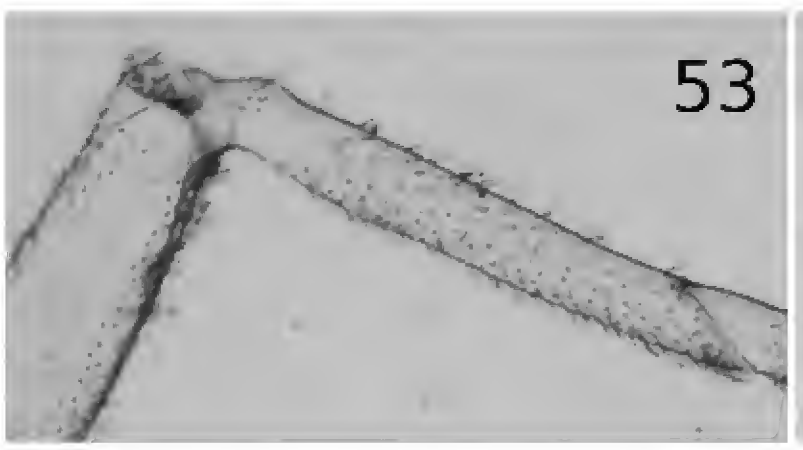
We have had little success in amplifying DNA from *Cloeon* specimens and so some larvae and adult associations have not yet been confirmed. The larvae of *C.* spBlackSpot remain unknown, but we are confident the larva *C.* spBigGill is equivalent to *C.* spBlackSpot. *C.* spQ3 is only known from larvae, but may be equivalent to larvae of *C.* spRedStripe from the Northern Territory, as they only differ in the presence of a small pair of dark spots near the anterior margin of the pro- and mesonota. No larva is known or suspected for *C.* spYellow or *C. virens*, and the description of *C. nandirum* is too vague to be of use so these species are not included in the key.

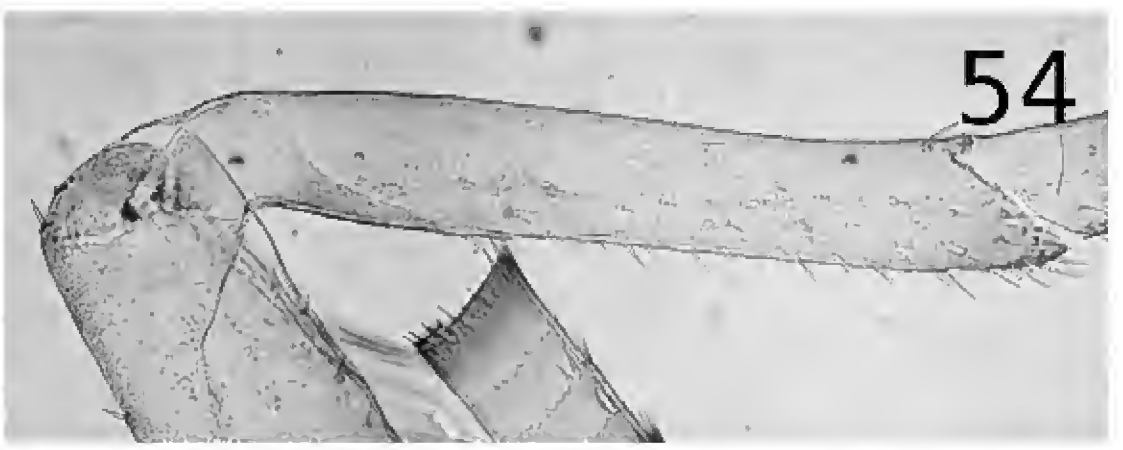
More than one species frequently occurs at the same locality, particularly in the Northern Territory and northern Queensland, so all specimens should be checked carefully.

Key to Larvae of *Cloeon*

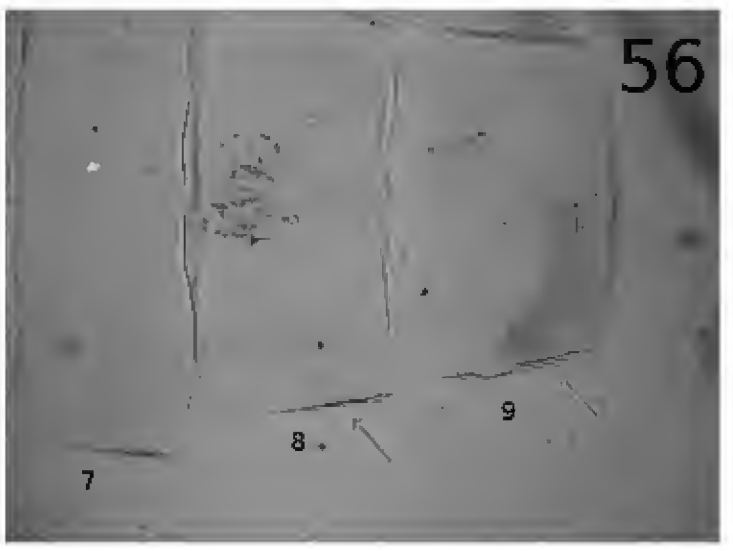
1a.Tasmania **2**
1b. Mainland Australia **3**

2a. Outer margin of mid- and hindtibiae with more than 5 robust setae (Fig 53) ..... ***C. paradieniense*** **(in part)**
2b. Outer margin of mid- and hindtibiae with 0-3 robust setae (Fig 54) ..... ***C. tasmaniae***

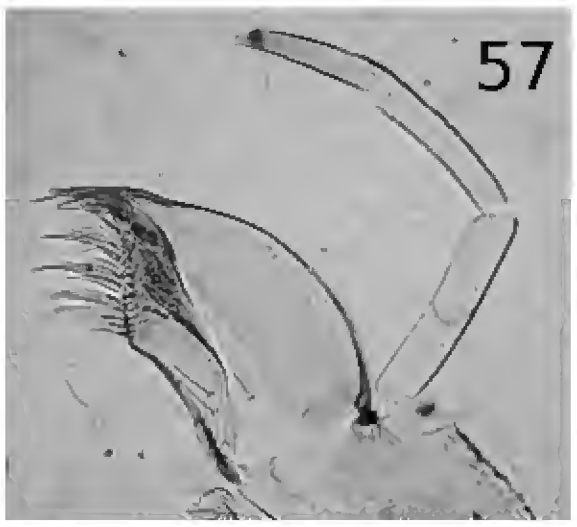


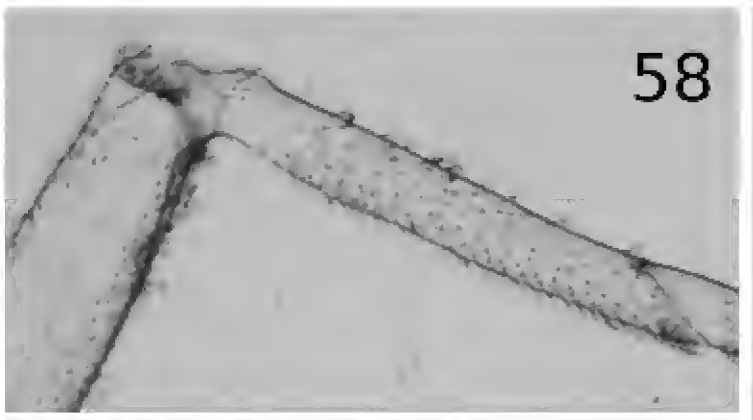


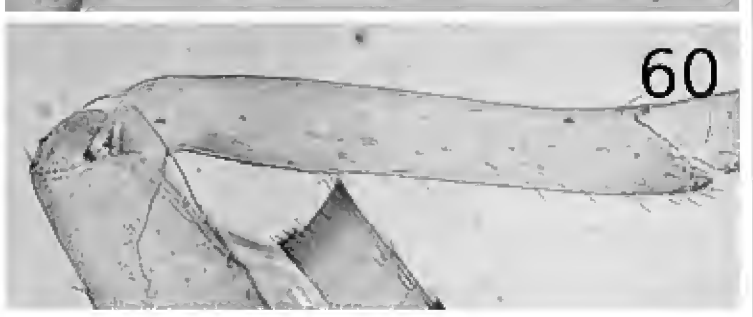
3a. Abdominal segments with lateral spines on 5 or 6 – 9 (Fig 55); widespread in all states **4**
3b. Abdominal segments usually with lateral spines only on segments 8-9, occasionally with 1 or 2 spines on segment 7 (Fig 56); northern Australia **5**

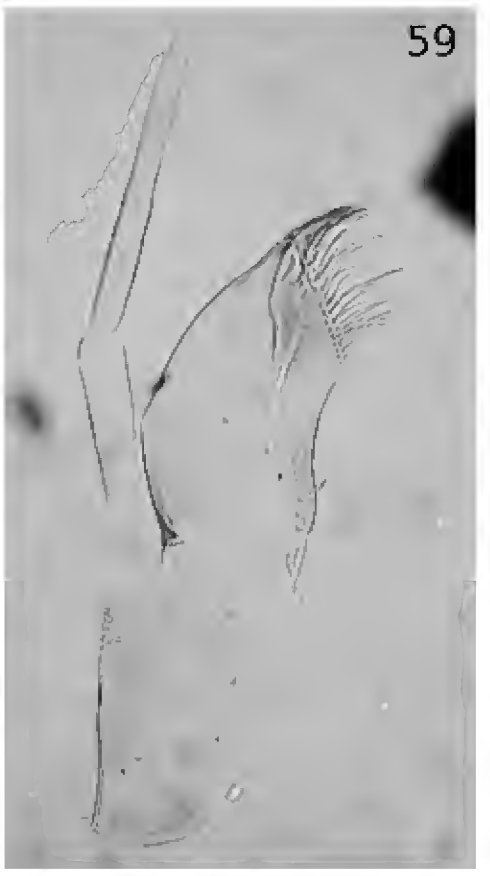


4a. Maxillary palps distinctly 3 segmented (Fig 57); outer margin of mid- and hindtibiae with more than 5 robust setae (Fig 58); South Australia, Victoria, New South Wales, Australian Capitol Territory, southeast Queensland? ..... ***C. paradieniense*** **(in part)**
4b. Maxillary palps usually distinctly 2 segmented (Fig 59), occasionally with an indistinct separation in segment two; outer margin of mid- and hindtibiae with 0 – 5 robust setae (as in Fig 60); widespread ..... ***C. fluviatile***



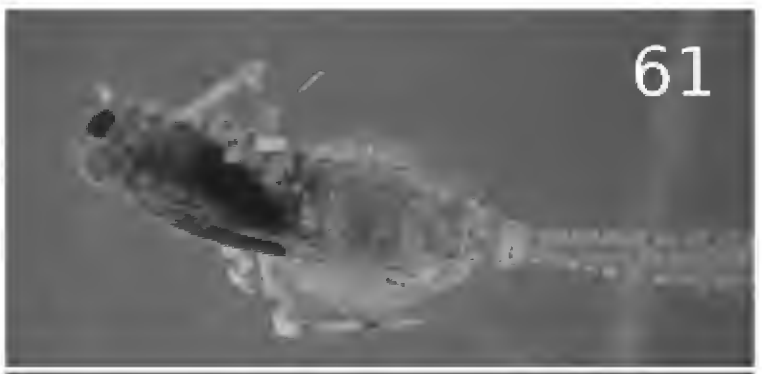


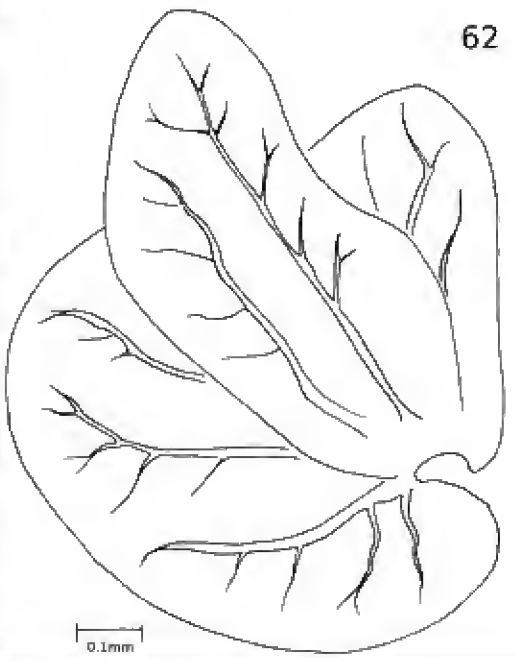


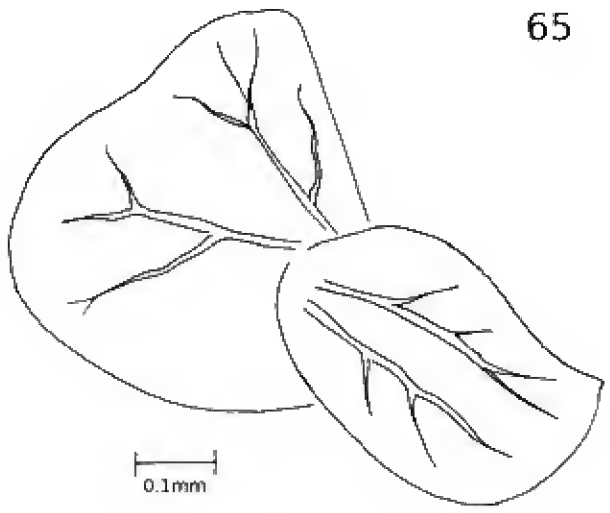


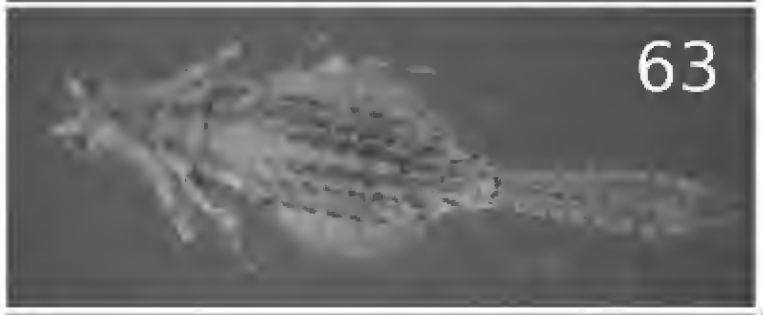
5a. Northern Queensland ….....**6**
5b. Northern Territory, northern Western Australia ….....**7**

6a. Gills extremely large (~0.8mm long, around 3 abdominal segment lengths) (Fig 61, 62); sterna of female larvae with distinct longitudinal red stripes ventrally (Fig 63); pro- and mesonotum without distinct pair of dark spots near anterior margin (Fig 61) ….....***C.* spBigGill**
6b. Gills 'normal' size (~0.4 – 0.5mm) (Fig 64, 65); sterna without distinct markings or with small median red spot on posterior margins (Fig 64b); pronotum and mesonotum usually with pair of submedian dark spots near anterior margin (Fig 64) ….....***C.* spQ3**

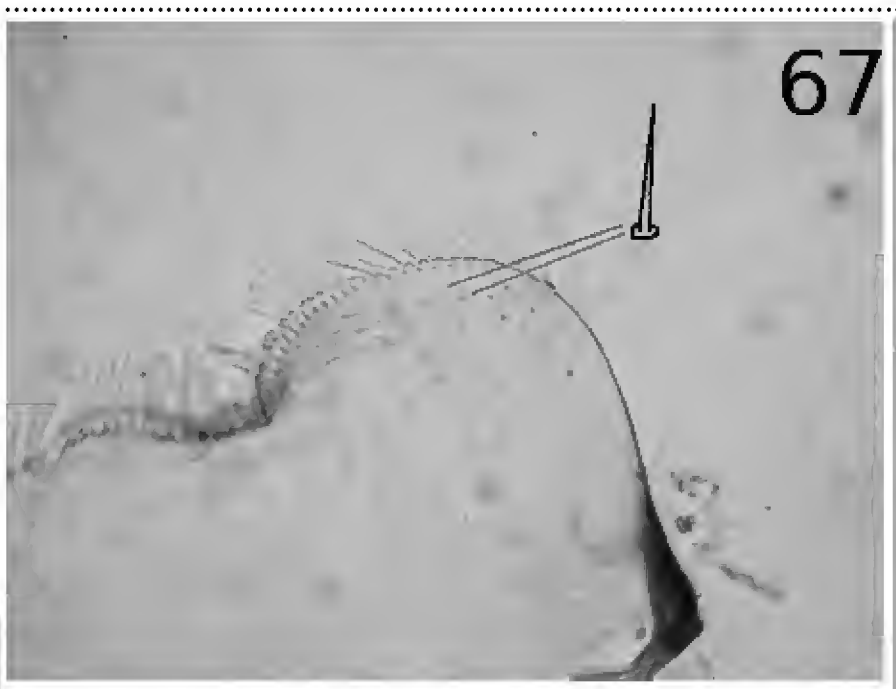


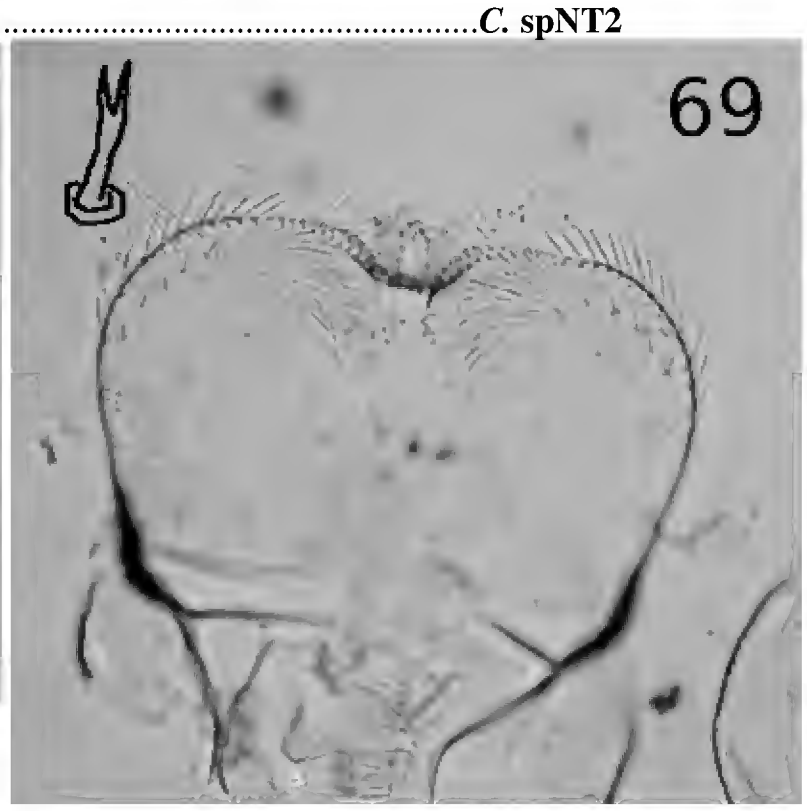


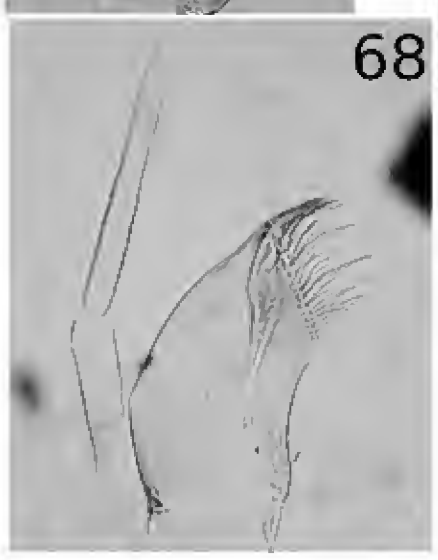




7a. Maxillary palps distinctly 3 segmented (Fig 66); ventral row of submarginal robust setae on labrum simple (Fig 67) .....***C.* spRedStripe**
7b. Maxillary palps 2 segmented (Fig 68); median 1 or 2 setae in ventral row of submarginal robust setae on labrum apically bifid (Fig 69) ….....***C.* spNT2**







Offadens Lugo-Ortiz & McCafferty

Diagnosis: Labrum with small U shaped notch; maxillary palps two segmented; labium with glossae and paraglossae subequal; hind wingpads present; tibiae and tarsi without arc of long fine setae; tarsal claws with single row of denticles and <0.5X length of tarsi; gills with single lamellae and present on segments 1 – 7; terminal filament subequal in length to cerci.

Distribution: Widespread in running waters.

Species Composition: *O. baddamsae* (Harker) [=Genus 2 spMV6, = *Edmundsiops instigatus* Lugo-Ortiz & McCafferty], *O. baddamsae* (Harker) Tasmania, *O. confluens* (Harker) [=Genus 1 spMV5], *O. frater* (Tillyard) [=Genus 1 sp8], *O. hickmani* (Suter) [=Genus 2 spMV3, in part], *O. instigatus* (Lugo-Ortiz & McCafferty), *O. sobrinus* Lugo-Ortiz & McCafferty [=Genus 1 sp7], *O. soror* (Ulmer) [=Genus1 WAsp1], *O.* G2Armidale1, *O.* G1ARR1, *O.* spEumerella, *O.* sphickmani-HighAltitude, *O.* sphickmani-LowAltitude, *O.* sphickmani-Gippsland, *O.* sphickmani-Otways, *O.* sphickmani-NNSW, *O.* sphickmani-CedarCreek, *O.* sphickmani-ManningR, *O.* spNariel, *O.* spSnowy, *O.* G2spMV1, *O.*G2spMV1-Timbarra, *O.* G2spMV2, *O.* G2sp9, *O.* G1spWA2, *O.*G4sp1, *O.* G1spMV4, *O.* G5sp2, *O.* sp16, *O.* sp17, *O.* sp18, *O.* sp20, *O.* spRingarooma

Discussion: Based on combined and separate analyses, both molecular and morphological characters show *Offadens* and *Edmundsiops* should be synonymised as *Offadens s.l.* is a lineage within *Edmundsiops*. *Offadens* is the older name and has priority.

DNA analyses show extensive divergence within morphological species previously identified. *O. hickmani*, for example is composed of 8 distinct mtDNA lineages based on sequencing >100 specimens. Sequences of two nuclear markers show these lineages to be reproductively isolated. A single individual has been found with a nuclear haplotype from a different mtDNA lineage, with which it was sympatric. Most of the mtDNA lineages are geographically isolated, but *O. sphickmani*Low Altitude is quite widespread throughout southern NSW and VIC and can be sympatic with either *O. sphickmani* HighAltitude or *O. sphickmani*Otway and *O. sphickmani*NNSW is sympatic with *O. sphickmani*ManningR. *O. sphickmani*ManningR and *O. sphickmani*CedarCreek are known only from single individuals at this time.

All species occurring in Tasmania appear to be different from species occurring on the mainland based on molecular evidence. The amount of divergence indicates this separation occurred several million years ago and predates the isolation of Tasmania from the mainland by rising sea levels at the end of the Pleistocene.

In other morphologically defined species, there are fewer cryptic species and most are Tasmania/mainland pairs. Other cryptic pairs include *O. frater* / *O.* spSnowy, *O.* spRingarooma / *O.* spNariel, In other cases, such as *O.* spRingarooma/ *O.*spNariel vs *O. frater*/*O.* spSnowy, the larvae appear identical but the adult stages differ.

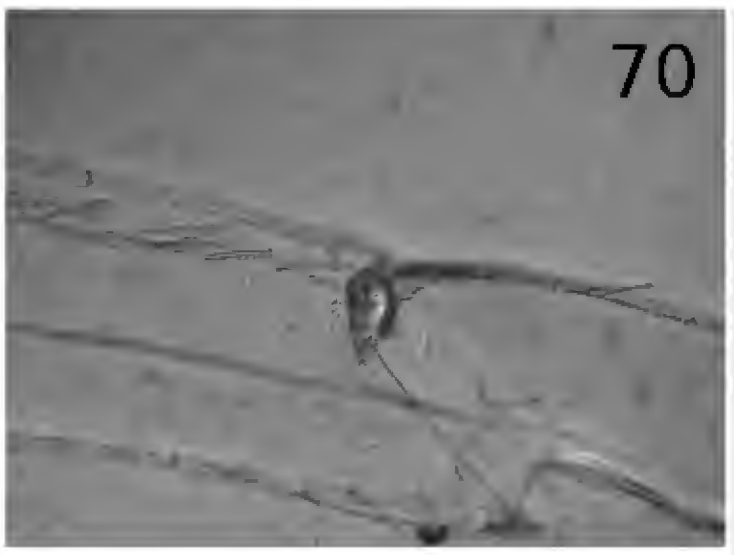
Offadens spG2MV1 has two divergent mtDNA lineages, one of which is only known from the Timbarra River in eastern Victoria. We have not found any morphological characters to separate these groups. Even within the 'normal' *O.* spG2MV1 there is considerable divergence among populations. This species tends to occur at higher altitudes so there are few chances for migration among populations. Populations on Mt. Buller, VIC, for example, differ from the population around Falls Creek, VIC by 4 – 5%. Haplotypes of the nuclear marker ITS1 are shared among these populations, as well as with the Timbarra R population. It is unclear if these shared haplotypes are evidence of reproduction among populations via male dispersal or if this is a case of incomplete lineage sorting. Additional, more variable, nuclear markers or the use of allozymes may clarify the status of these populations. For the time being, we consider there to be two species.

The larva previously known as Genus 2 spMV6 was recently found to be equivalent to *Edmundsiops baddamsae* (Harker)(Webb and Suter, 2010a), and is now treated as *Offadens baddamsae* **n.comb**. Furthermore, the holotype of *E. instigatus* was found to be equivalent to *O. baddamsae* and so the species were synonymised (Webb and Suter 2010a). Webb and Suter (2010a) also examined material from the type locality of *Offadens confluens* (Harker) and found the larva to be equivalent to a more restrictive concept of Genus 1 spMV5 *sensu* Suter (1997).

Key to *Offadens* Species

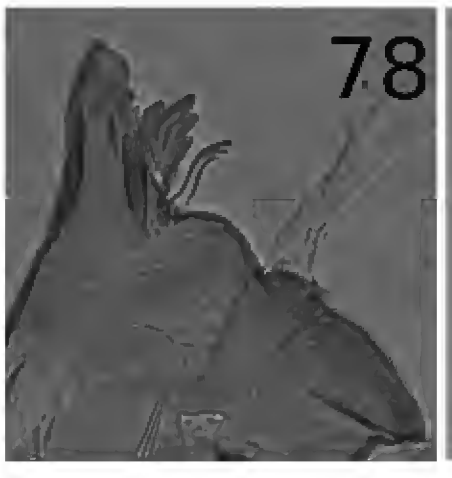
1a. Outer margin of tibiae and tarsi with distinct row of long, darkly coloured robust setae that are ~0.4X the width of the tibia (Figs 70, 71); SE QLD to Tasmania ..... *O. hickmani* complex **23**

1b. Tibae and tarsi variable, usually with row of short robust setae on tibiae <0.3X width of tibia (Figs 72, 73), but row may be absent; widespread **2**

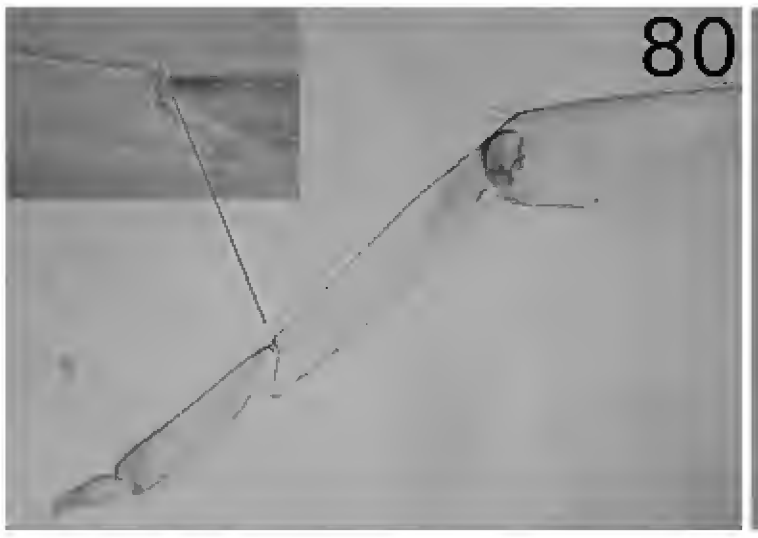


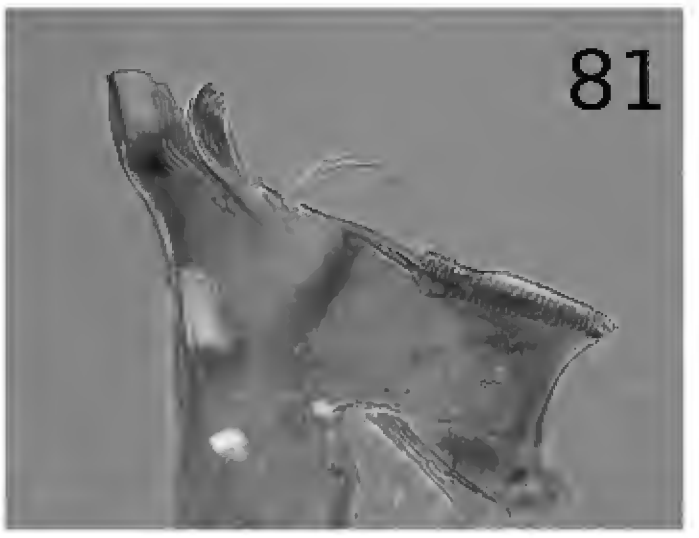
2a. Right mandible with distinct tooth-like process between base of prostheca and molar (Fig 74); prostheca simple (Fig 75) ….....**13**
2b. Right mandible usually flat (Fig 76), but may be distinctly rounded (Fig 77), never with a tooth like projection; prostheca simple or forked (Fig 76) **3**

3a. Right mandible with distinct hump between prostheca and molar (Fig 78); prostheca simple ….....***O.* spArmidale1**
3b. Right mandible flat between prostheca and molar (Fig 79); prostheca simple (as in Fig 78) or forked (Fig 79) ….....**4**

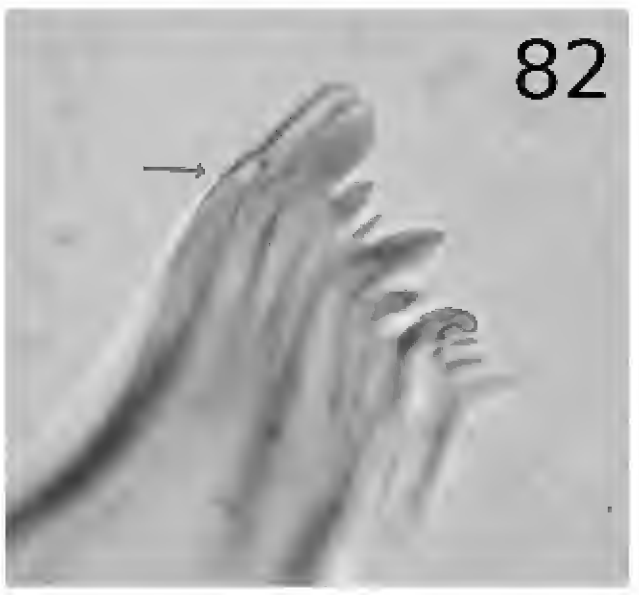


4a. Prostheca simple (as in Fig 78); subapical pair of setae on femora and apical seta on tibiae extremely long (Fig 80); N Qld ….....*O.* G5sp1 complex **5**
4b. Prostheca forked (Fig 81); subapical setae of femora and apical seta of tibiae usually much shorter, or absent; if setae are long, then not in southern New South Wales and Victoria ….....**6**

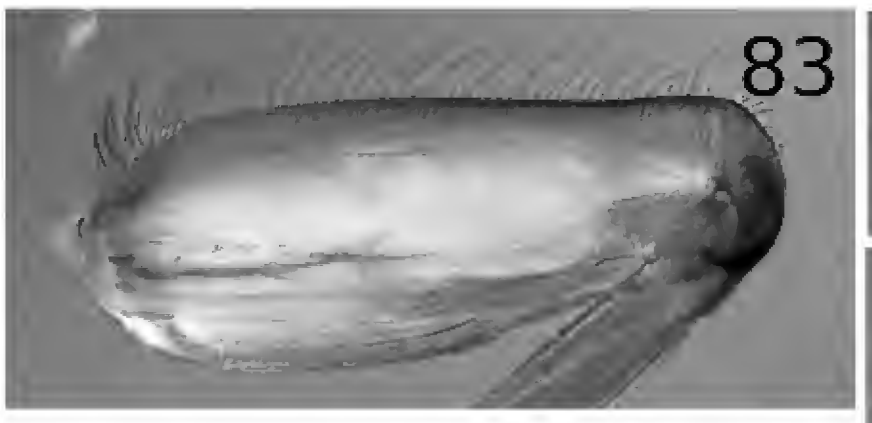


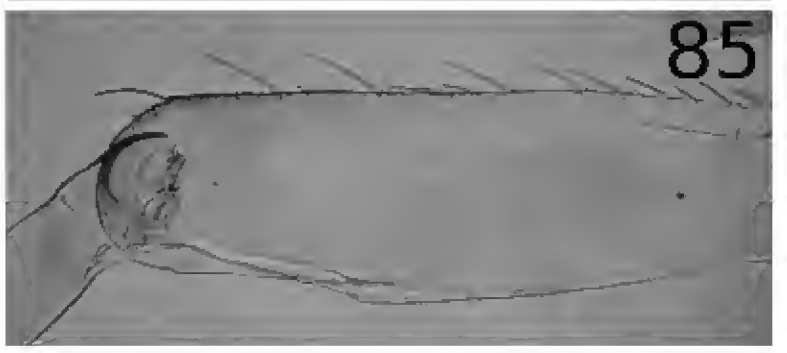


5a. Right mandible with small lateral tooth on outer incisor (Fig 82); body length 2.6-4.1 mm (mean = 3.19 mm); mostly occur in sandy bottomed streams ….....***O.* sp16 and *O.* sp18**
5b. Right mandible without small lateral tooth on outer incisor; body length 3.1 – 5.6 mm (mean = 4.26 mm); mostly occurs in rocky, fast flowing stream in the Atherton Tablelands, but also known from lowland streams ….....***O.* sp17**

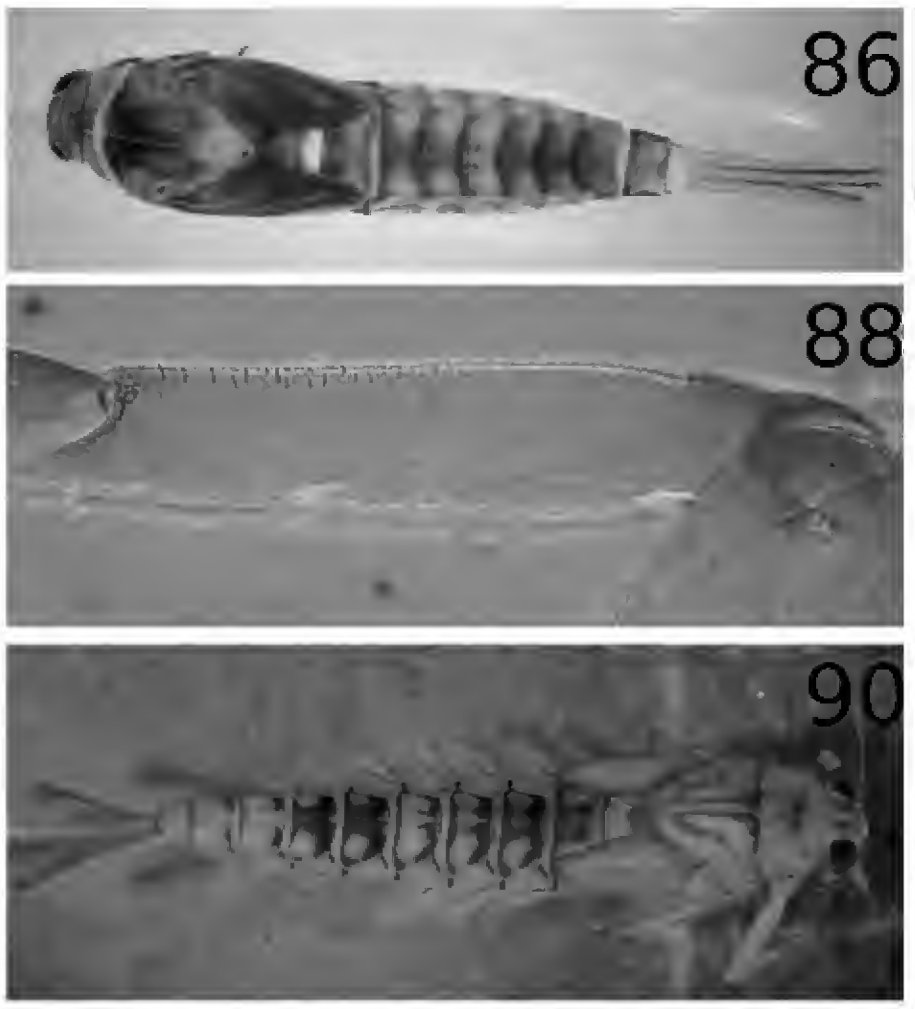


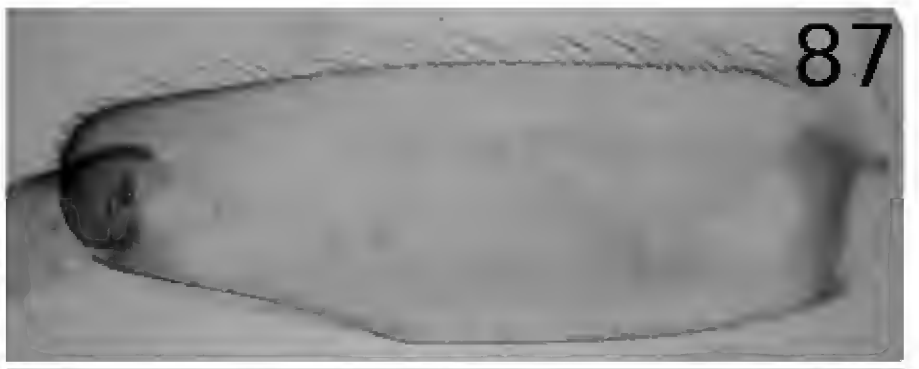
6a. Dorsal margin of femora densely lined with more than 40 long hairlike setae (Fig 83) **7**
6b. Dorsal margin of femora with less than 30 somewhat stout setae (Figs 84, 85) **11**





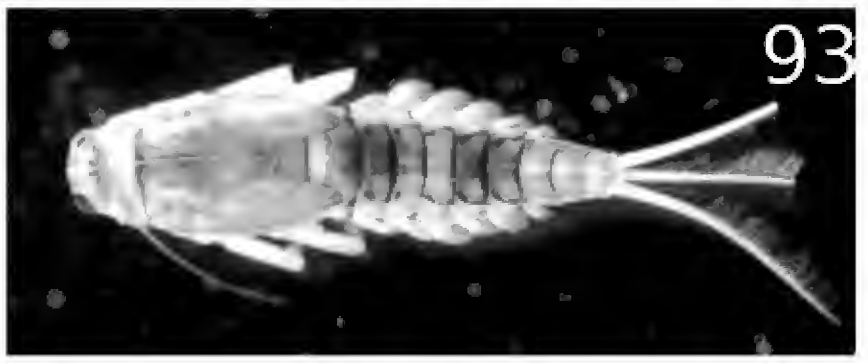
7a. Abdominal terga 1-9 mostly brown, slightly paler laterally but without distinct spots, tergum 10 usually white (Fig 86); sternum usually dark brown; apex of femora with long robust setae (Fig 87); subapical setae absent on both femora and tibiae (Fig 87, 88), tibiae with distinct row of robust setae on outer margin (Fig 88); high altitude streams of NSW and Vic ….....***O.*sp MV1** complex **28**
7b. Abdominal terga with a distinct colour pattern, usually dark with distinct pale spots and some segments paler than others (Figs 89,90,91); sternum variable; subapical setae of tibiae and femora variable ….....**8**



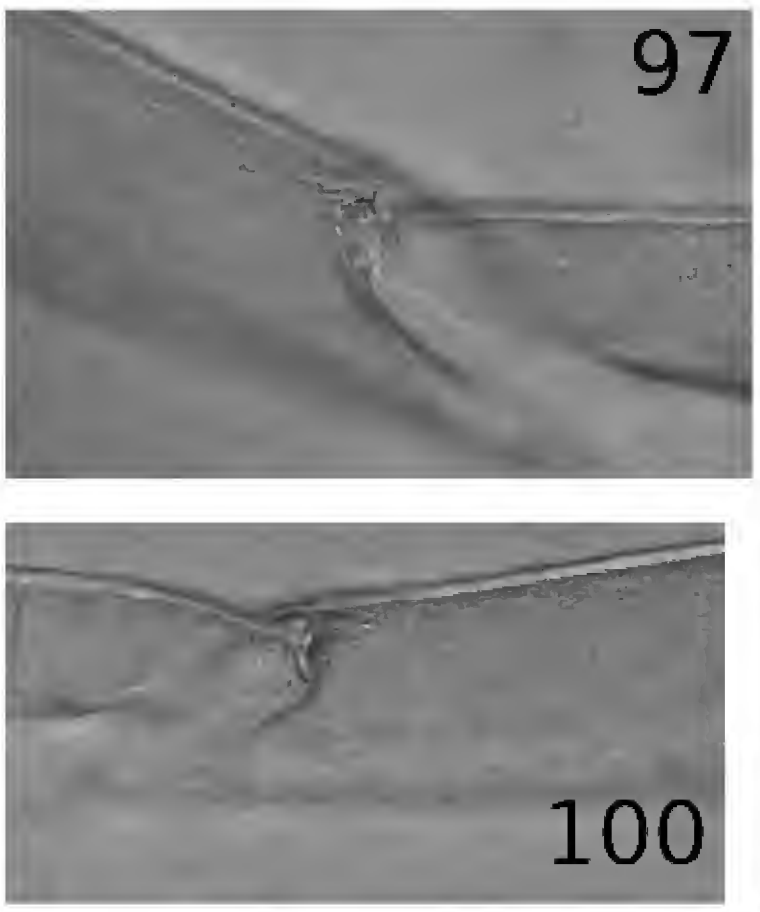


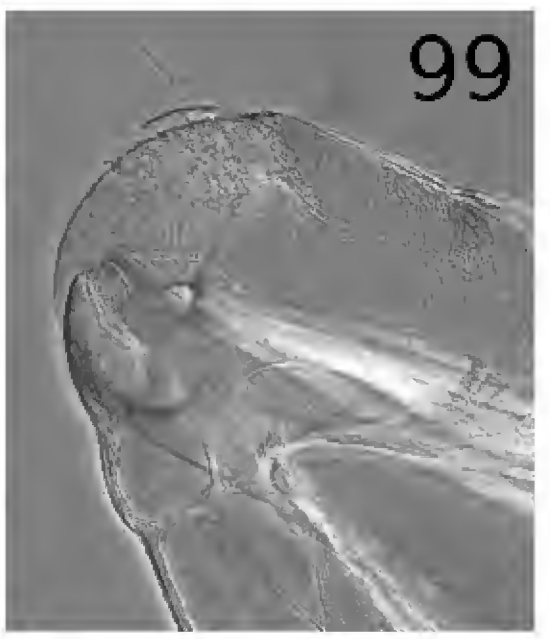
8a. Apical setae of femora long (Fig 92); abdominal segments with a transverse reddish band on the posterior margins, wider medially (Figs 91, 93); Queensland .....***O.* G5sp2**

8b. Apical setae of femora short and globular (Fig 94); abdominal segments with uniformly narrow transverse band or without band (Figs 95, 96) ….....**9**



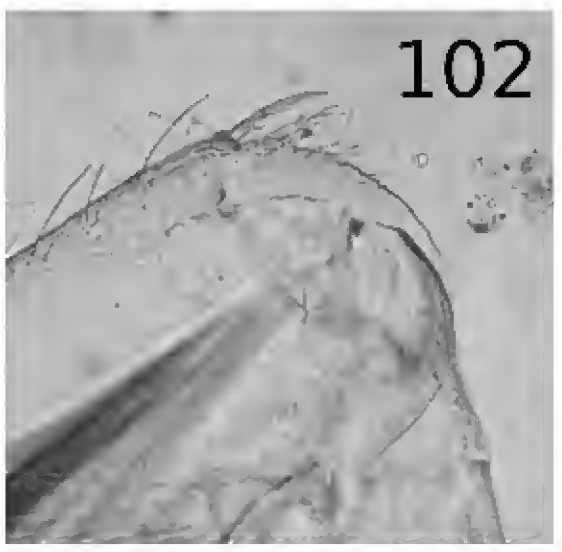
9a. Femora and tibiae without subapical seta, but a continuation of tibial row of short, somewhat cloven, robust setae (Fig 97); forewing pads with distinct pale transverse band in freshly collected specimens (Fig 98); sternum usually dark brown posteriorly; New South Wales, Victoria ….....***O.* G2spMV2**
9b. Femora and tibiae with subapical setae (Fig 99, 100) and tibia with distinct fringe of fine setae; forewing pads unicolorous (Fig 101); sternum usually pale; SE Queensland to Tasmania ….....**10**

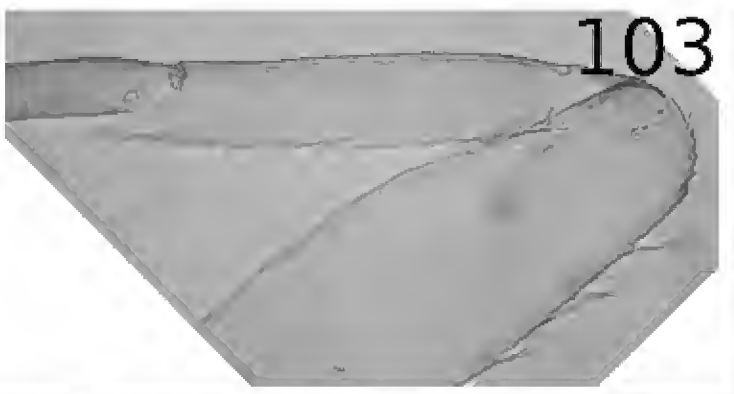


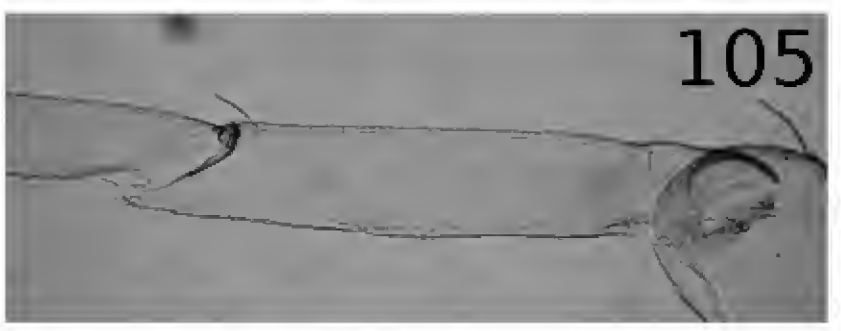


10a. Tasmania .....***O. baddamsae* Tasmania**
10b. SE Queensland to Victoria ..... ***O. baddamsae* mainland**

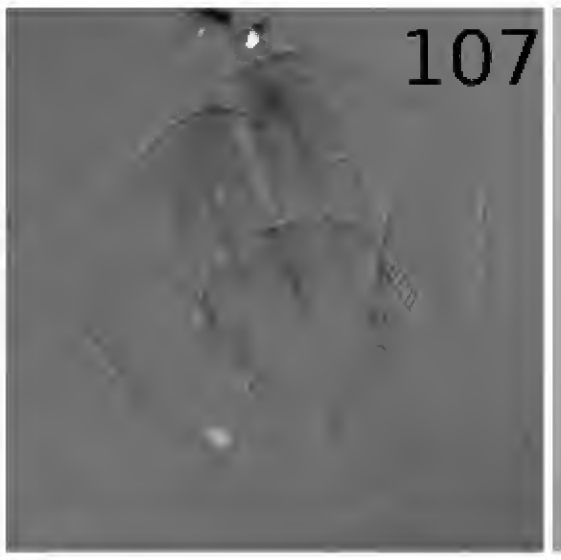
11a. Northern Queensland; apical setae on femora long (Fig 102); outer margin of tibiae with distinct row of short robust setae (Fig 103); abdominal terga 1-8 usually mostly brown, tergra 9-10 white (Fig 104) ….....….....***O.* G4sp1**
11b. New South Wales and Victoria; apical setae on femora short and globular, subapical setae very long (Fig 105); outer margin of tibiae with only very small robust setae (Fig 105) ….....**12**

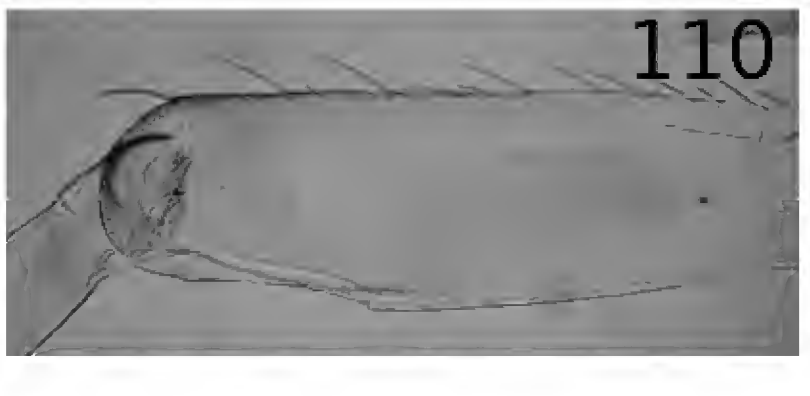






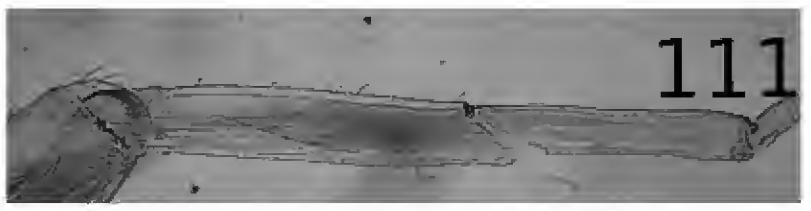
12a. Colour pattern as in Fig 106; labial palps with distinct medial projection on segment 2 (Fig 107); tarsal claws with subapical seta; subapical seta of tibiae either absent or small ….....***O.* G2sp9**
12b. Colour pattern as in Fig 108; labial palps with only small medial extension on segment 2 (Fig 109); tarsal claws without subapical seta; tarsi and femora with very long subapical setae (Figs 105, 110) .....***O.* G1sp4**

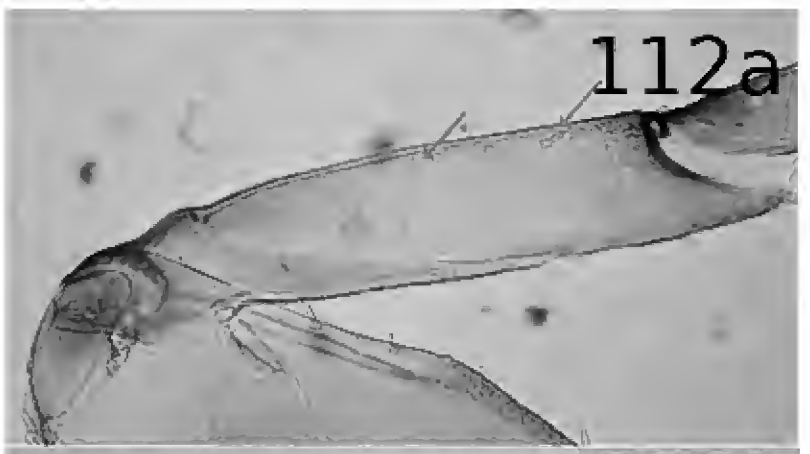


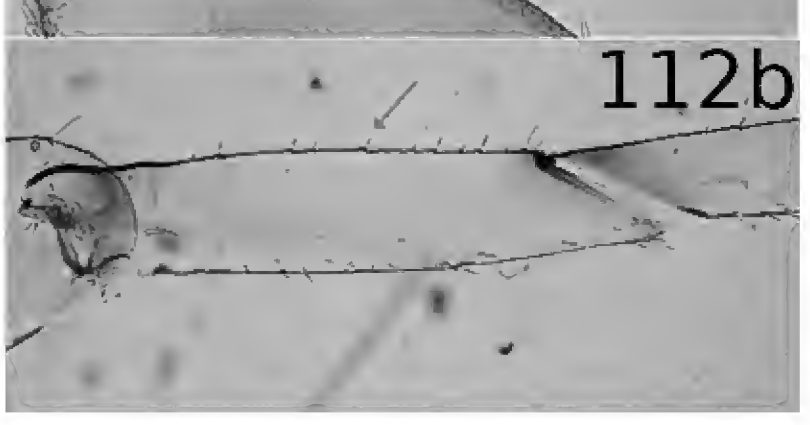


13a. Western Australia **14**
13b. Not Western Australia **15**

14a. Tibiae with row of long robust setae on outer margin (Fig 111); NW Western Australia .....***O.* G1spWA2**
14b. Tibiae with row of short robust setae on outer margin (Fig 112a), occasionally with long robust setae (Fig 112b); throughout western Western Australia .....***O. soror***



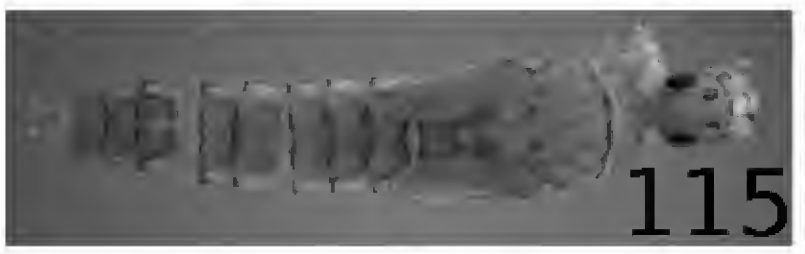


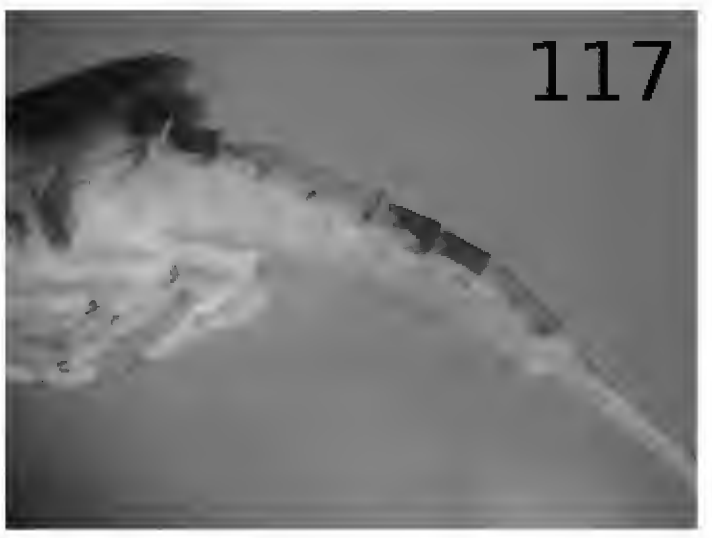


15a. North Qld or NT; abdominal terga as in Figs 113, 114; subapical setae on tarsal claws present .....***O.* ARR1**
15b. SE Queensland to Tasmania **16**

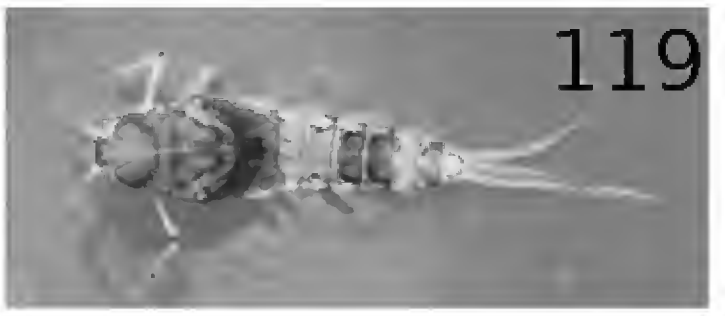
16a. Tasmania **17**
16b. Mainland **18**

17a. Abdominal terga of later instar larvae with adult pattern of two red transverse dashes on posterior margins and laterally with distinct red spot on segment 2 and sometimes segment 7 – 9 (Fig 115) .....***O. frater***
17b. Abdominal terga without red markings on posterior margin and laterally with relatively diffuse red markings on all or most segments (as in Figs 116, 117) .....***O.* spRingarooma**



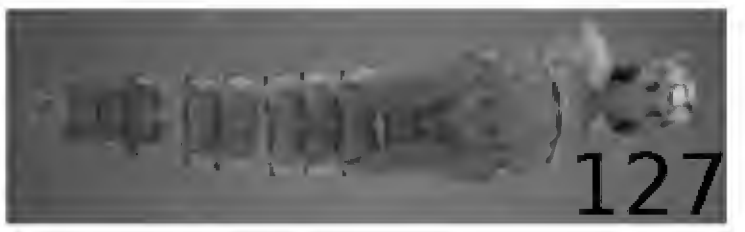


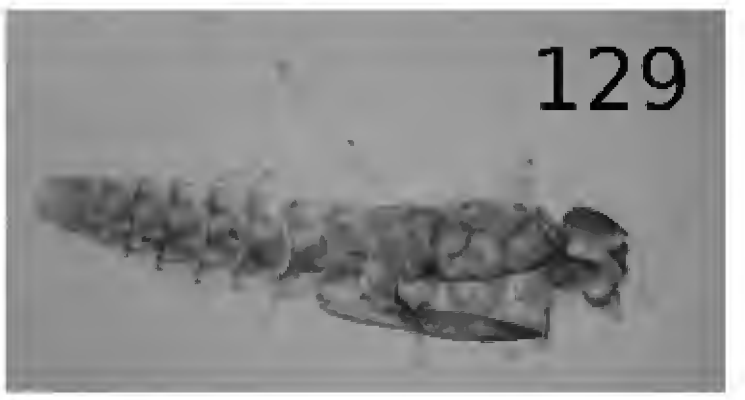
18a. Colour pattern of fresh specimens as in Fig 118; tarsal claws with subapical seta; SE South Australia, SW Victoria .....***O.* spEumerella**
18b. Colour pattern variable (Fig 119, 120, 121); tarsal claws without subapical setae **19**



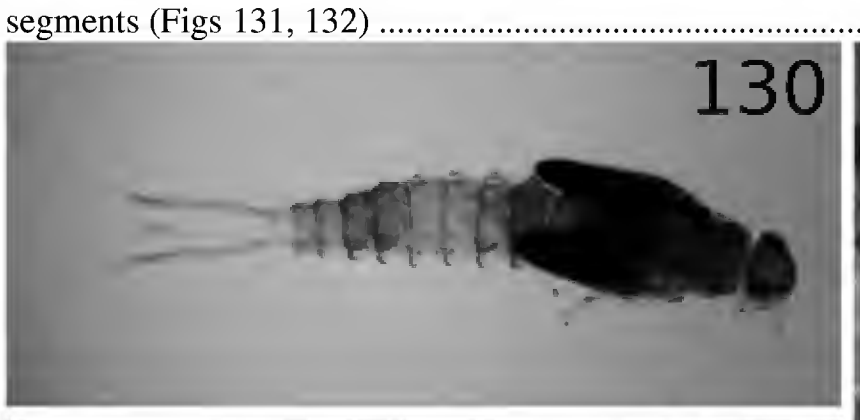
19a. Tibiae without robust setae on outer margin (Fig 122); colour pattern as in Figs 123, 124; New South Wales .....***O. sobrinus***
19b. Tibiae with a row of robust setae on outer margin (Fig 125); SE Queensland to South Australia **20**

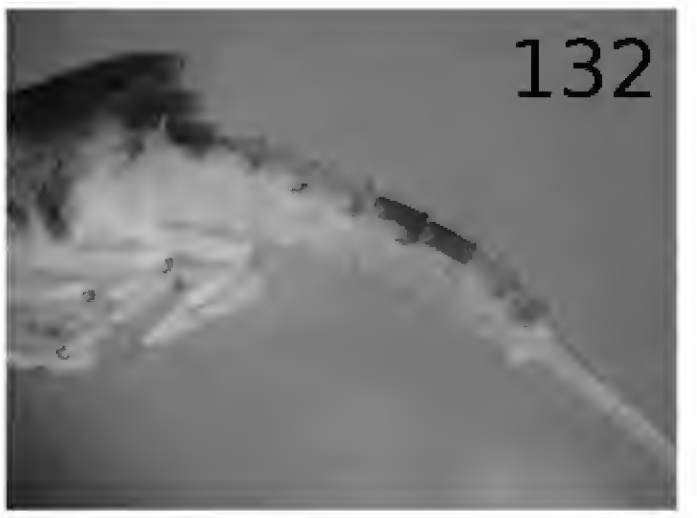
20a. Abdominal terga of male larvae with usually with contrasting light and dark segments (Fig 126); females sometimes less contrastingly coloured (as in Fig 127); usually occurs in streams with moderate to fast flows; Victoria, New South Wales ….....**21**
20b. Abdominal terga 1 – 9 mostly brown, with a colour pattern as in Fig 128 and 129; gills often much longer than wide, but variable within and among populations; usually occurs in slower flowing lowland streams; SE Queensland to South Australia **22**



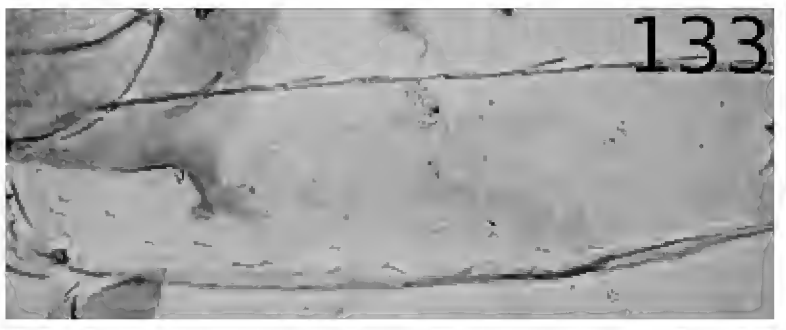


21a. Abdominal terga of later instar larvae with adult pattern of two red transverse dashes on posterior margins and laterally with distinct red spot on segment 2 and sometimes segment 7 – 9 (Fig 130) …..*O.* **spSnowy**
21b. Abdominal terga without red markings on posterior margin and laterally with relatively diffuse red markings on all or most segments (Figs 131, 132) .....*O.* **spNariel**





22a. Femora with robust setae relatively sparsely distributed on anterior surface (Fig 133); outer incisor of mandibles with small lateral tooth (as in Fig 134); foretibiae with subapical seta; SE Queensland to South Australia ….....*O. confluens*
22b. Femora very densely covered with robust setae (Fig 135); outer incisor of right mandible without small lateral tooth (Fig 136); foretibiae without subapical seta; known only from a single adult male with associated exuviae from near Bathurst, New South Wales ….....*O.* **sp20**



23a. Tasmania ..... ***O.hickmani***
23b. Victoria **24**
23c. New South Wales, possibly SE Queensland **26**

24a. Otways and Grampians ..... ***O.hickmani* Otways, *O.hickmani* LowAltitude**
24b. Gippsland ..... ***O.hickmani* Gippsland**
24c. Great Dividing Range not in Gippsland **25**

25a. Usually at altitudes >800m, but may occur lower in small fast flowing streams..... ***O.hickmani* HighAltitude**
25b. Usually at altitudes <800m, but may occur higher in slower, warmer streams ..... ***O.hickmani* LowAltitude**

26a. South of Hunter River **27**
26b. North of Hunter River ..... ***O.hickmani* NNSW, *O.hickmani* ManningR, *O.hickmani* CedarCreek**

27a. Usually at altitudes >800m, but may occur lower in small fast flowing streams..... ***O.hickmani* HighAltitude**
27b. Usually at altitudes <800m, but may occur higher in slower, warmer streams ..... ***O.hickmani* LowAltitude**

28a. Timbarra River, VIC, and possibly other streams on eastern side of Great Dividing Range in northern Victoria ***O*.spMV1-Timbarra**
28b. New South Wales, western side of Great Dividing Range in Victoria ..... ***O*. spMV1**

Platybaetis Müller-Liebenau

Diagnosis: Head prognathous with distinct right angle between labrum and frons; labrum with small U shaped notch; maxillae two segmented; paraglossae much wider than glossae; hind wingpads absent; tarsal claws with single row of denticles and < 0.5X length of tarsi; gills with single lamellae and present on segmens 2-7; terminal filament vestigial.

Distribution: fast flowing streams in Northern Territory and Northern Queensland.

Species Composition: *P. gagadjuensis*

Discussion: Both morphological and molecular characters show the Australian species to be more closely related to *Pseudocloeon* than true *Platybaetis*. It is unclear at this time if a new genus should be established or if *P. gagadjuensis* should be placed in *Pseudocloeon*. A global analysis of the relationships among *Pseudocloeon* and other *Baetis*-complex genera is required as the current concept of *Pseudocloeon* is polyphyletic.

Pseudocloeon Klapálek

Diagnosis: Labrum with small U shaped notch; maxillae two segmented; paraglossae much wider than glossae; hind wingpads absent; tarsal claws with single row of denticles and < 0.5X length of tarsi; gills with single lamellae and present on segmens 2-7; terminal filament subequal in length to cerci.

Distribution: mostly northern Australia, but *P. hypodelum* occurs as far south as Victoria.

Species Composition: *P. hypodelum* Lugo-Ortiz & McCafferty [=Genus 3 sp4], *P. inconspicuum* Lugo-Ortiz & McCafferty [= Genus 3 sp2], *P. plectile* Lugo-Ortiz & McCafferty [=Genus 3 sp3], *P.* sp5.

Discussion: The generic concept of *Pseudocloeon* was revised by Lugo-Ortiz *et al.* (1999), but most workers believe this concept to be polyphyletic. The type species of *Pseudocloeon*, *P. kraepelini* Klapálek was described from Java and has been reported from Australia. It is likely however, that the Australian report was based on a misidentification as no adults of Australian *Pseudocloeon* have been described and species identification of adult baetids can rarely be done with certainty.

Two species were described by Lugo-Ortiz and McCafferty (Lugo-Ortiz *et al.* 1999) based on single specimens collected from Mt. Kosciuszko at an elevation of 1700m. Extensive collections have yielded no additional specimens from this area, and being a tropical group, are not expected to occur in high altitude alpine streams. It is likely that the labels on the type specimens are incorrect and the specimens actually came from northern Queensland or the Northern Territory.

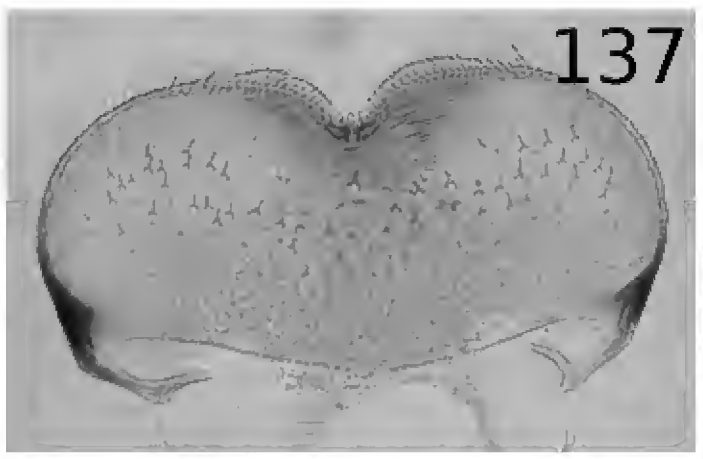
Pseduocloeon inconspicuum has two divergent lineages in northern Queensland based on mtDNA, although we have only sequenced a small number of specimens. Although we only have a small number of specimens, there appears to be a consistent size difference between the two forms, but we have not yet found other morphological differences. For the time being, we are keying these out separately. We have not yet sequenced specimens from the Northern Territory.

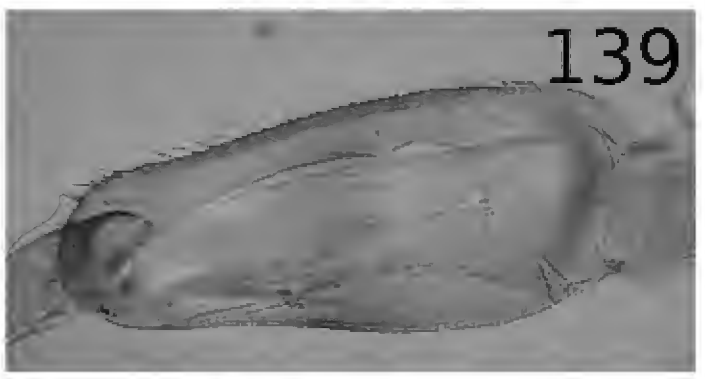
Pseudocloeon hypodelum was initially described from Queensland, but it is now known from as far south as Victoria, where it is abundant in warmer rivers such as the lower Ovens and Broken Rivers. *P.* sp5 is unique in Australia in having a short labrum with many sharp robust setae on the dorsal surface; in other characters, it is similar to the PNG species *P. xeniolum* Lugo-Ortiz & McCafferty and they may be closely related

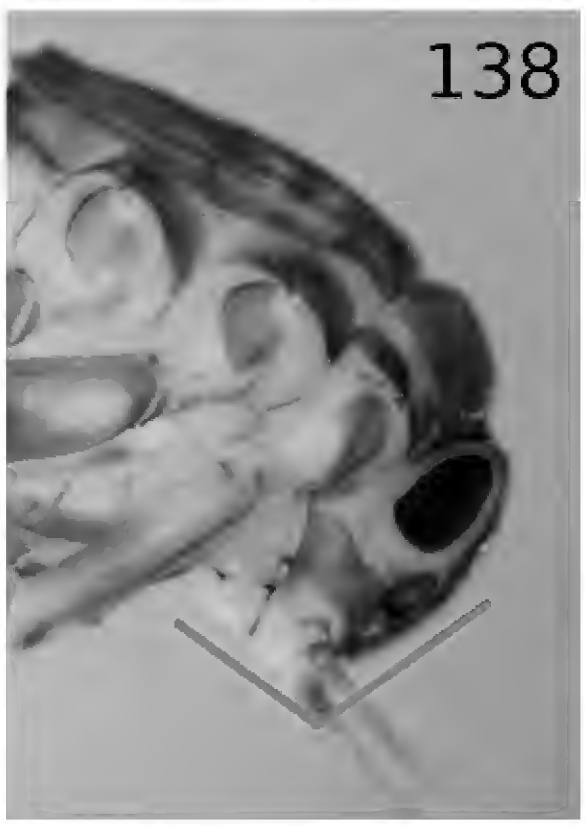
.

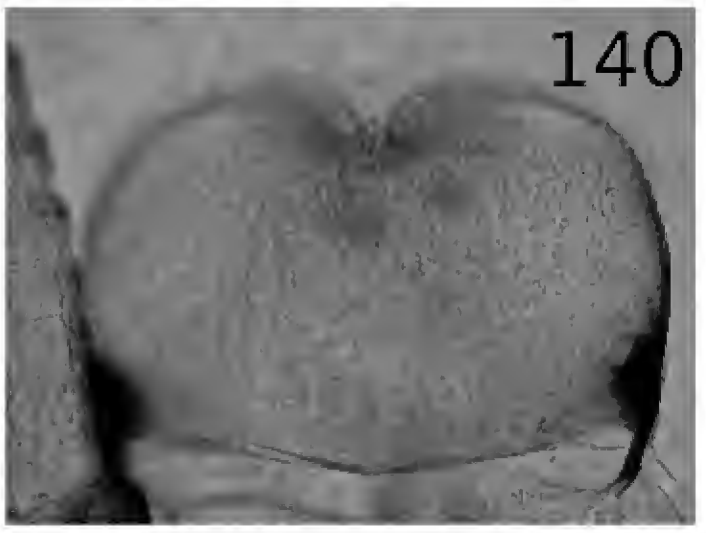
Key to Species of *Pseudocloeon*

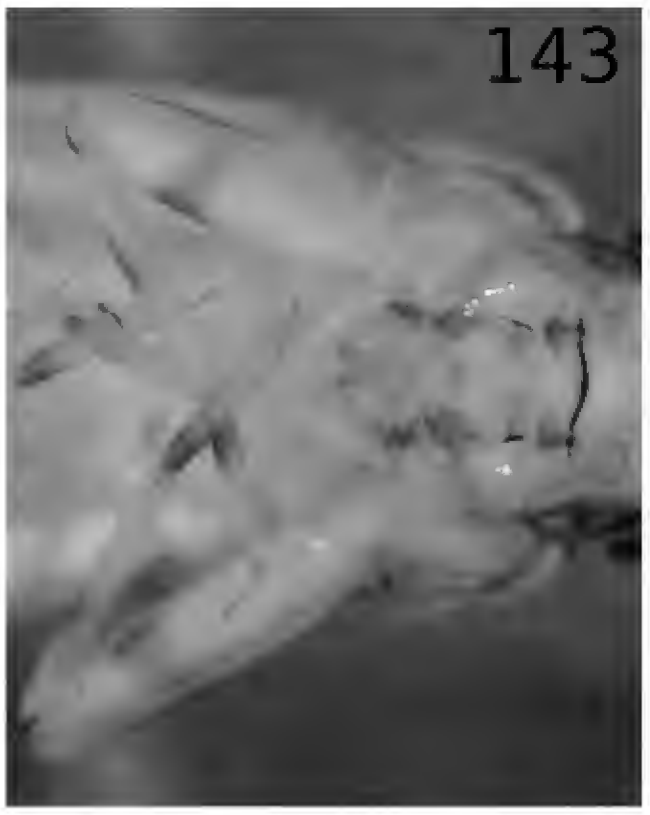
1a. Labrum much wider than long and covered with many sharply pointed robust setae (Fig 137); head with distinct right angle between frons and labrum, appearing pug-nosed (Fig 138); femora broad (Fig 139) ……***P.* sp5**
1b. Labrum only slightly wider than long and never covered with sharp robust setae (Fig 140); head rounded in lateral view (Fig 141); femora broad (Fig 142) or narrow (Fig 143) ……**2**



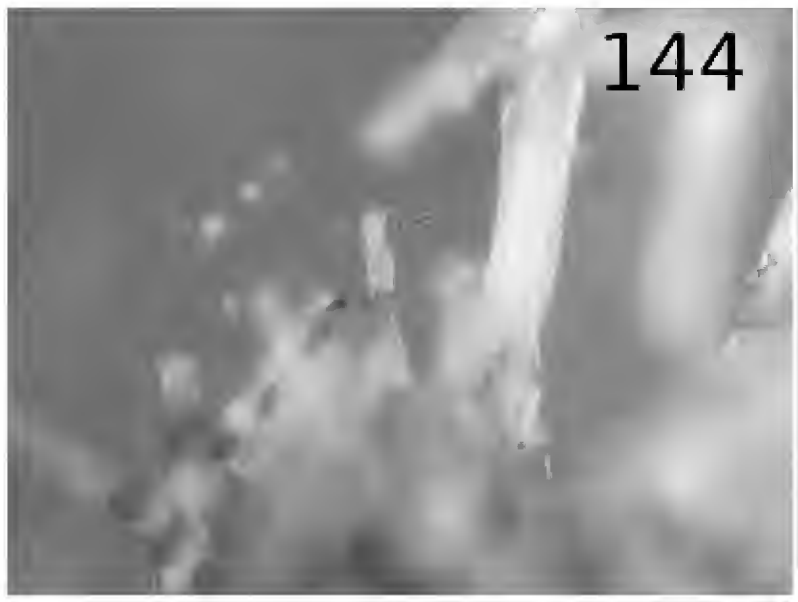


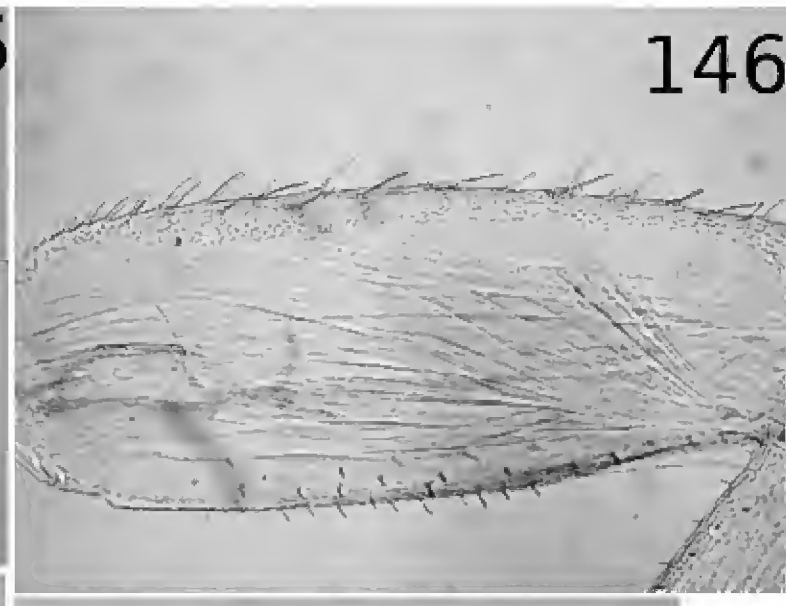


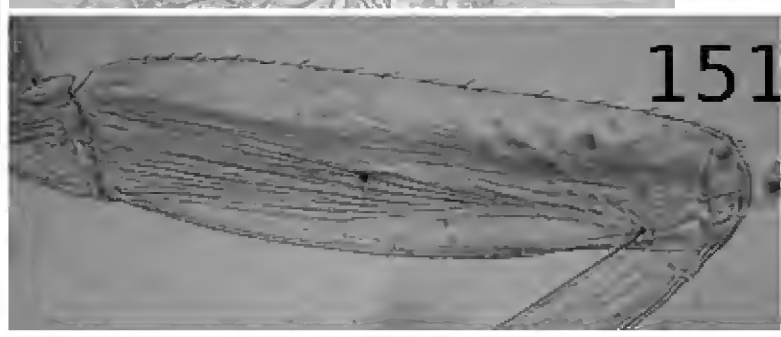


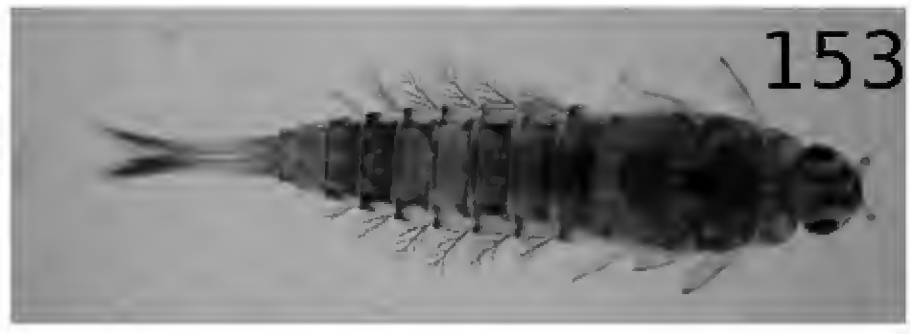


2a. Maxillary palps with distinct subapical excavation (Fig 144); labial palps with median swelling on segment 2 (Fig 145); femora with two rows of robust setae on dorsal margin (Fig 146) (most easily seen from a dorsal view); colour pattern mostly brown with tergum 10 pale posteriorly and with a small median pale spot (Fig 147) ……***P. inconspicuum* complex ..3**
2b. Maxillary palps without subapical excavation (Fig 148); labial palps with narrow thumb-like process on segment 2 (as in Fig 149, 150); femora with a single row of robust setae on dorsal margin (Fig 151); colour pattern variable (Figs 152, 153) ……**4**



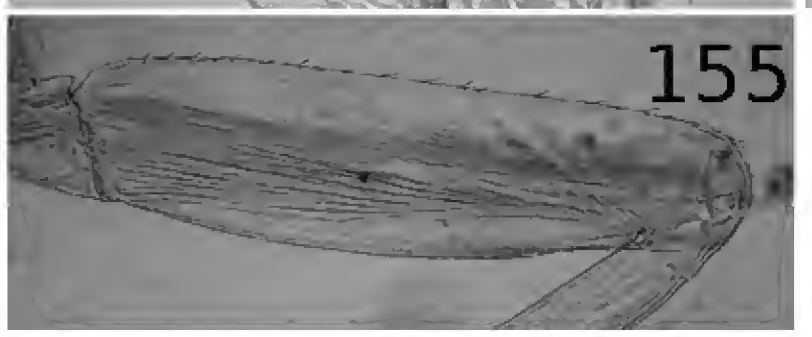






3a. Up to 3.2mm; gills without dark tracheae .....***P. inconspicuum* small**
3b. Up to 4.8mm; gills with dark tracheae .....***P. inconspicuum* large**

4a. Labial palp relatively narrow and with long thumb-like process on segment 2 (Fig 154); femora narrow (Fig 155); abdominal tergum 9 with pale posterior border and medial stripe (Fig 152); northern Western Australia, Northern Territory, Queensland, New South Wales, Victoria .....***P. hypodelum***
4b. Labial palp relatively robust and with short process on segment 2 (as in Fig 156); femora broad (Fig 157); northern Queensland and Northern Territory .....***P. plectile***



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